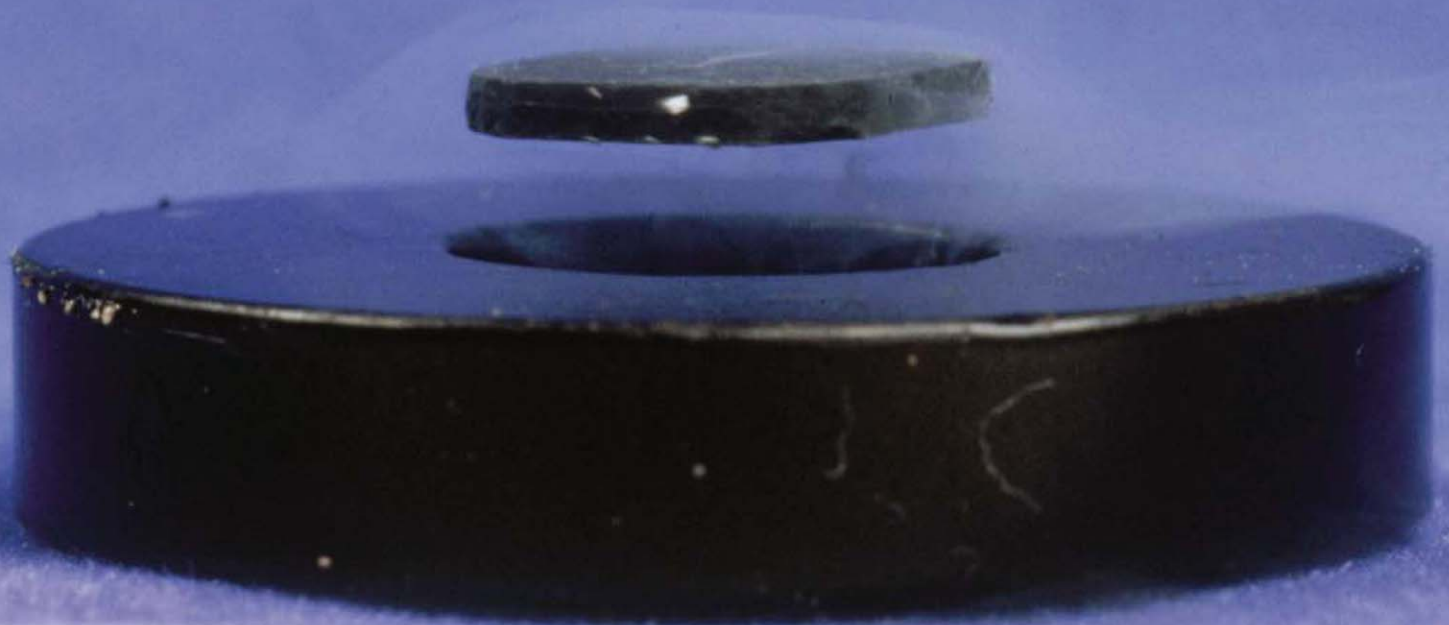


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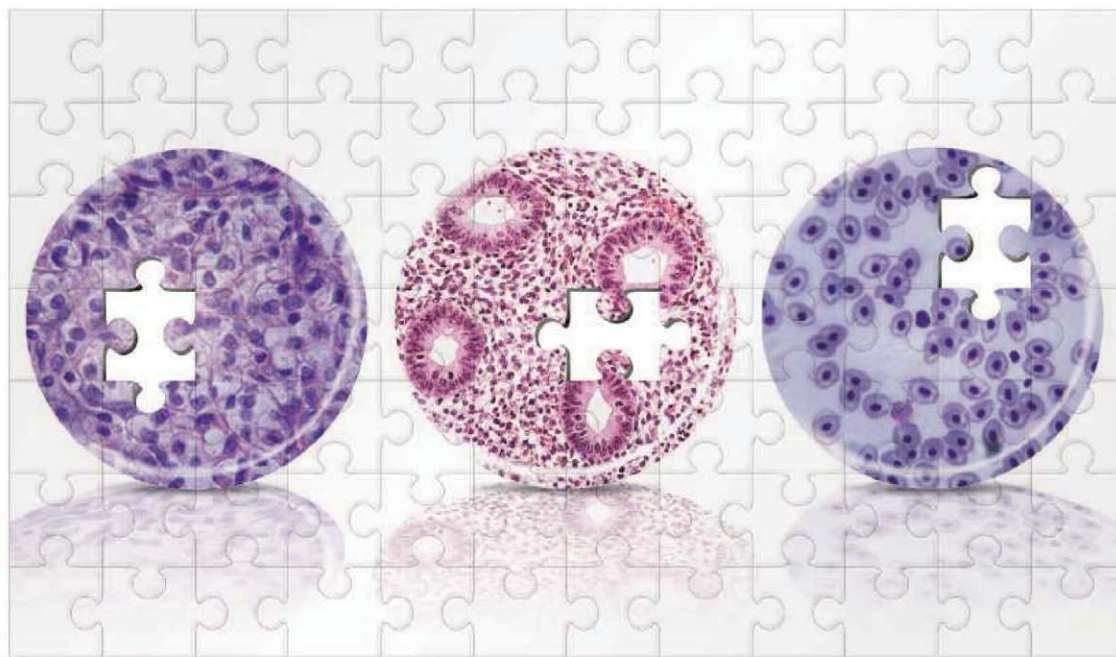
(McGraw-Hill Dictionary of Scientific and Technical Terms, 4th Edition).

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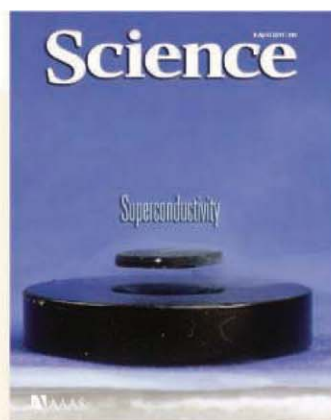
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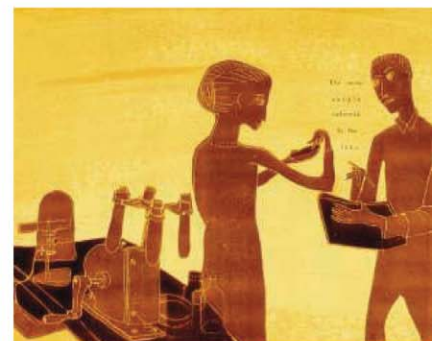
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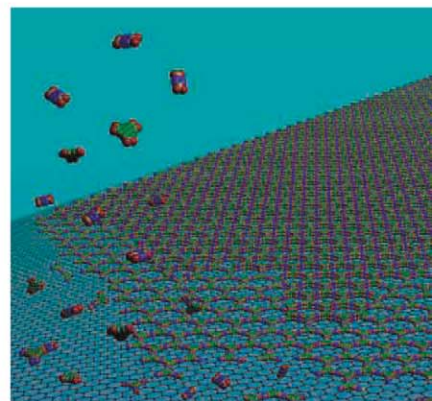
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**Trans-Endocytosis of CD80 and CD86:
A Molecular Basis for the Cell Extrinsic
Function of CTLA-4**

O. S. Qureshi et al.

An inhibitory T cell receptor acts by stripping activating ligands off dendritic cells.

10.1126/science.1202947

**Neuronal GPCR Controls Innate Immunity
by Regulating Noncanonical Unfolded
Protein Response Genes**

J. Sun et al.

Two nematode worm neurons “smell” disease and promote resistance to pathogens.

10.1126/science.1203411

Observation of Orbital Currents in CuO

V. Scagnoli et al.

Resonant x-ray scattering is used to detect microscopic loop currents within the plane of cupric oxide.

10.1126/science.1201061

**Orbital-Independent Superconducting
Gaps in Iron-Pnictides**

T. Shimajima et al.

Bulk photoemission studies of iron pnictides suggest a role for orbital fluctuations in creating the superconducting state.

10.1126/science.1202150

**Venus’s Southern Polar Vortex Reveals
Precessing Circulation**

D. Luz et al.

Observations with the Venus Express Orbiter reveal complex polar atmospheric dynamics.

10.1126/science.1201629

TECHNICALCOMMENTS

**Comment on “Calcareous Nannoplankton
Response to Surface-Water Acidification
Around Oceanic Event 1a”**

S. J. Gibbs et al.

Full text at www.sciencemag.org/cgi/content/full/332/6026/175-b

**Response to Comment on “Calcareous
Nannoplankton Response to Surface-Water
Acidification Around Oceanic Event 1a”**

E. Erba et al.

Full text at www.sciencemag.org/cgi/content/full/332/6026/175-c

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Genetic fingerprints reveal movement of deadly bacterium from Europeans to native Canadians.

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The Curse of the Mummies’ Arteries

A new study traces the origins of heart disease in ancient Egypt.

<http://scim.ag/mummy-curse>

A Bacterium That Acts Like a Toothbrush

Oral microbe fights plaque buildup, could lead to development of better toothpaste.

<http://scim.ag/bug-tooth>

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**RESEARCH ARTICLE: Poly(ADP-Ribose) (PAR)
Binding to Apoptosis-Inducing Factor Is Critical
for PAR Polymerase-1–Dependent Cell Death
(Parthanatos)**

Y. Wang et al.

Poly(ADP-ribose) binds to apoptosis-inducing factor to trigger its release from mitochondria and induce cell death.

**RESEARCH ARTICLE: Confinement of Activating
Receptors at the Plasma Membrane Controls
Natural Killer Cell Tolerance**

S. Guia et al.

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S. Ugolini et al.

The responsiveness of activating NK cell receptors is determined by the distribution of inhibitory receptors.

**PERSPECTIVE: ATM Is a Redox Sensor Linking
Genome Stability and Carbon Metabolism**

A. Krüger and M. Ralser

By linking genome stability, the cell cycle, and carbon catabolism, ATM emerges as a central regulator of cancer cell metabolism.

**PERSPECTIVE: All Stressed Out
Without ATM Kinase**

J. J. P. Perry and J. A. Tainer

Oxidation activates ATM, allowing this kinase to mediate antioxidant responses.

**PRESENTATION: Proteomic Analysis
of Integrin Adhesion Complexes**

A. Byron et al.

A workflow for the proteomic analysis of integrin-associated complexes reveals ligand-specific adhesion networks.

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B. L. Benderly

Postdoc-turned-politician Peter Ferguson hopes to bring his scientific insight to Canada’s federal Parliament.

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**Q&A: Philip Phillips—A Roundabout
Approach to Superconductivity**

E. Pain

His unconventional training allowed theoretical condensed matter physicist Philip Phillips to tackle superconductivity using a novel and indirect approach.

<http://scim.ag/qaphillips>

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6 April issue: <http://scim.ag/stm040611>

**STATE OF THE ART REVIEW: Alzheimer’s Disease—
The Challenge of the Second Century**

D. M. Holtzman et al.

PODCAST

D. M. Holtzman and O. M. Smith

The first article in our State of the Art Review series explores the challenges of translating research advances into clinical treatments for Alzheimer’s disease.

**RESEARCH ARTICLE: Genital HIV-1 RNA Predicts
Risk of Heterosexual HIV-1 Transmission**

J. M. Baeten et al.

**PERSPECTIVE: HIV Transmission—Time for
Translational Studies to Bridge the Gap**

P. Anton and B. C. Herold

Genital HIV-1 RNA quantity predicts risk of heterosexual HIV-1 transmission independently of plasma HIV-1 concentration.

**RESEARCH ARTICLE: CD44-SLC1A2 Gene Fusions
in Gastric Cancer**

J. Tao et al.

One partner of a fusion gene found in gastric cancer, CD44-SLC1A2, may contribute to the tumor’s abnormal metabolism.

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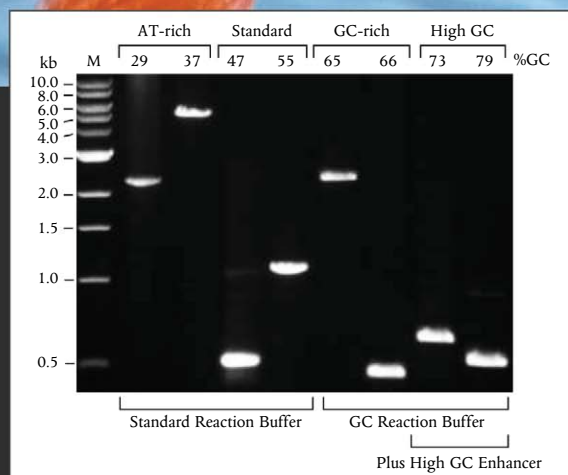
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<< Asteroseismology Delivers

Using asteroseismology—the study of stellar oscillations, it is possible to probe the interior of stars and to derive stellar parameters, such as mass and radius (see the Perspective by **Montgomery**). Based on asteroseismic data from the NASA Kepler mission, **Chaplin *et al.*** (p. 213) detected solarlike oscillations in 500 solar-type stars in our Galaxy. The distribution of the radii of these stars matches that expected from stellar evolution theory, but the distribution in mass does not, which challenges our knowledge of star formation rates, the mass of forming stars, and the models of the stars themselves. **Derekas *et al.*** (p. 216) report the detection of a triple-star system comprising a red giant star and two red dwarfs. The red giant star, instead of the expected solarlike oscillations, shows evidence for tidally induced oscillations driven by the orbital motion of the red dwarf pair. Finally, **Beck *et al.*** (p. 205) describe unusual oscillations from a red giant star that may elucidate characteristics of its core.

The Ribozyme World

In the hypothetical ancient “RNA world,” DNA’s chemical sister RNA played the central genetic and catalytic roles in biological processes. Implicit in such a system is the existence of an RNA polymerase ribozyme. Previous *in vitro* evolution and step-wise engineering created the R18 RNA polymerase ribozyme, which has limited polymerase activity (up to 14 nucleotides). **Wochner *et al.*** (p. 209; see the Perspective by **Yarus**) used the R18 ribozyme as a starting point to engineer and evolve further a new RNA polymerase ribozyme capable of synthesizing templates up to ~94 nucleotides in length with high fidelity. The improved ribozyme could synthesize a small self-cleaving hammerhead ribozyme.

Capturing 3D on Thin Film

Holograms store the phase and amplitude information of light bouncing off an object as an interference pattern in a photographic plate. A three-dimensional reconstruction of the object can be achieved by illuminating the hologram with light of the same wavelengths used to store the hologram. **Ozaki *et al.*** (p. 218) describe a color holographic technique based on the diffraction of surface plasmons propagating at the surface of a thin metal film. The surface plasmons were excited using white-light-angled illumination and the incident angle of illumination determined the color diffracted to the viewer.

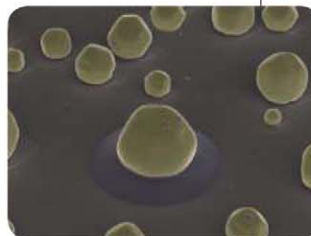
Burn, Baby, Burn

The summer of 2010 was a real scorcher in large parts of Eastern Europe. **Barriopedro *et al.*** (p. 220, published online 17 March) show that the heat wave was the greatest in the last 500

years. In western Russia alone, there were more than 55,000 heat-related deaths, extensive wildfires, an annual crop failure of ~25%, and a total economic loss of around 1% of Russia’s gross domestic product. Models indicate that less extreme heat waves are 5 to 10 times more likely over the next 40 years.

Understanding Intergranular Films

Intergranular films appear at grain and phase boundaries in polycrystalline materials and at free surfaces. They play an important role in the mechanical and functional properties of many systems. **Baram *et al.*** (p. 206; see the Perspective by **Harmer**) studied the interfaces between gold droplets and a sapphire surface with a partial coating of anorthite glass beads. The anorthite formed the basis of a nanometer-thick intergranular film, which caused a decrease in the gold-sapphire interfacial energy. Unlike other artificially made conventional thin films, these films did not break up during equilibration, which may provide a useful design criterion in thin-film technology.



Probing Suspensions

Catalysis often takes place in complicated biphasic environments, with molecules in pressurized gas or liquid phase briefly contacting catalyst

surfaces and then emerging chemically transformed from the interface. In contrast, studies of catalyst-substrate binding interactions are often restricted to simpler conditions, such as bare catalyst surfaces under near vacuum. **Tedsree *et al.*** (p. 224) now show that nuclear magnetic resonance spectroscopy can reveal signatures of binding under realistic conditions—aqueous suspensions of noble metal nanoparticles—that strongly correlate with catalytic activity.

Minor Spliceosome Gains Stature

For nearly two decades after the discovery that eukaryotic messenger RNA precursors contain noncoding sequence interruptions called introns, it was assumed that all introns and all spliceosomes (the cellular machinery that excises introns) were alike. Then rare, outlier introns were identified that violated certain sequence rules and were excised by special spliceosomes. Because these so-called “minor” introns account for less than 1% of introns in human cells, their impact on biology has been unclear (see the Perspective by **Pessa and Frilander**). Now, **He *et al.*** (p. 238) and **Edery *et al.*** (p. 240) report that mutational disruption of an RNA component specific to the minor spliceosome (U4atac snRNA) causes severe developmental defects in humans. Individuals with homozygous mutations in this RNA are afflicted with a rare inherited disorder called microcephalic osteodysplastic primordial dwarfism type I (MOPD I) or Taybi-Linder syndrome (TALS) and die in the first few years of life.

Continued on page 147



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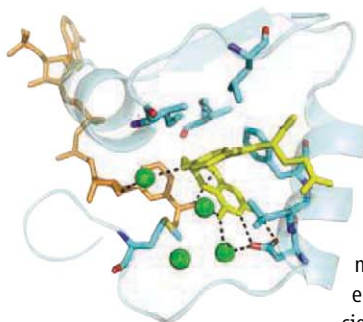
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Eosinophils and Metabolism

Alterations in macrophage activation in adipose tissue are associated with metabolic disease. In lean mice and humans, macrophages with an immunoregulatory phenotype (termed alternatively activated macrophages, or AAMs) predominate, whereas AAM numbers decrease and the numbers of proinflammatory macrophages increase when metabolic control is lost. **Wu *et al.*** (p. 243, published online 24 March; see the Perspective by **Maizels and Allen**) now show that eosinophils, an immune cell type classically associated with parasitic infections and allergy, regulate the number of AAMs present in adipose tissue in mice. Eosinophils were detected in adipose tissue where they produced interleukin-4, a cytokine that promotes the AAM phenotype. Eosinophil deficiency in mice was associated with a decrease in AAMs in adipose tissue and increased body fat and blood glucose levels in mice fed a high-fat diet. In contrast, genetic or parasite-induced increases in eosinophil numbers resulted in improved metabolic read-outs in mice fed a high-fat diet.

Orienting Organic Networks on Graphene

Covalent organic frameworks (COFs), like metal organic frameworks, have organic linking groups, but the structure-directing groups in COFs are typically fused aromatic rings rather than metal atoms. Although COF materials are robust, they tend to form as insoluble powders that are difficult to process into films. **Colson *et al.*** (p. 228) show that a COF, COF-5, formed from 4-phenylenebis(boronic acid) and 2,3,6,7,10,11-hexahydroxytriphenylene under mild solvothermal conditions, produced crystalline films on different types of single-layer graphene. The layers were vertically aligned, an orientation that may prove useful in organic photovoltaic applications.



Dynamic Chemistry

Conformational fluctuations can mediate ligand binding and be rate-limiting for enzyme turnover. However, the role of active-site dynamics is debated. **Bhabha *et al.*** (p. 234) generated a mutant of *Escherichia coli* dihydrofolate reductase with significantly impaired hydride transfer but with no significant changes in the structure or electrostatic environment of the active site. Millisecond time-scale fluctuations were abrogated in the active site of the mutant, suggesting that the active site could not sample the higher-energy conformations conducive to transition-state formation efficiently and thus failed to promote the chemical reaction.

The Real World Is Messy

A common theme in recent social psychology research studies is the testing of laboratory-derived theories and mechanisms in so-called ecologically valid settings (that is, in field experiments). **Stapel and Lindenberg** (p. 251) show that environmental signs of disorder, such as uncollected trash at a train station or cars parked askew on a sidewalk, are sufficient to induce bystanders to desire orderliness. The consequences are that these bystanders elected to sit further from minorities when asked to fill in a survey and donated less of their payments (for participating in the survey) to help immigrants and the homeless. Laboratory-based experiments suggested that the desire for order was fulfilled by an increased propensity toward classification, which includes stereotyping.

Invasion of the Body Snatchers

The whitefly, *Bemisia tabaci*, ranks in the world's top 15 worst-invasive alien species. Like many other insects, it plays host to several bacteria that affect its physiology in various ways, including enhancing availability of nutrients, viral resistance, and changing sex ratios. The bacterium *Rickettsia bellii* was first observed in 2000 in a few whitefly specimens collected from the southwestern United States, but in fewer than 80 generations (in just 6 years) it achieved 97% infection rates. **Himler *et al.*** (p. 254; see the Perspective by **Jiggins and Hurst**) found that almost all daughter insects are infected if their mothers are infected and that there is little male-to-female transmission. But, as might be surmised from their spread, infection is not a bad thing for the insects—infected whiteflies double their fecundity, develop faster into adults, and produce more females.

CREDIT: BHABHA ET AL.

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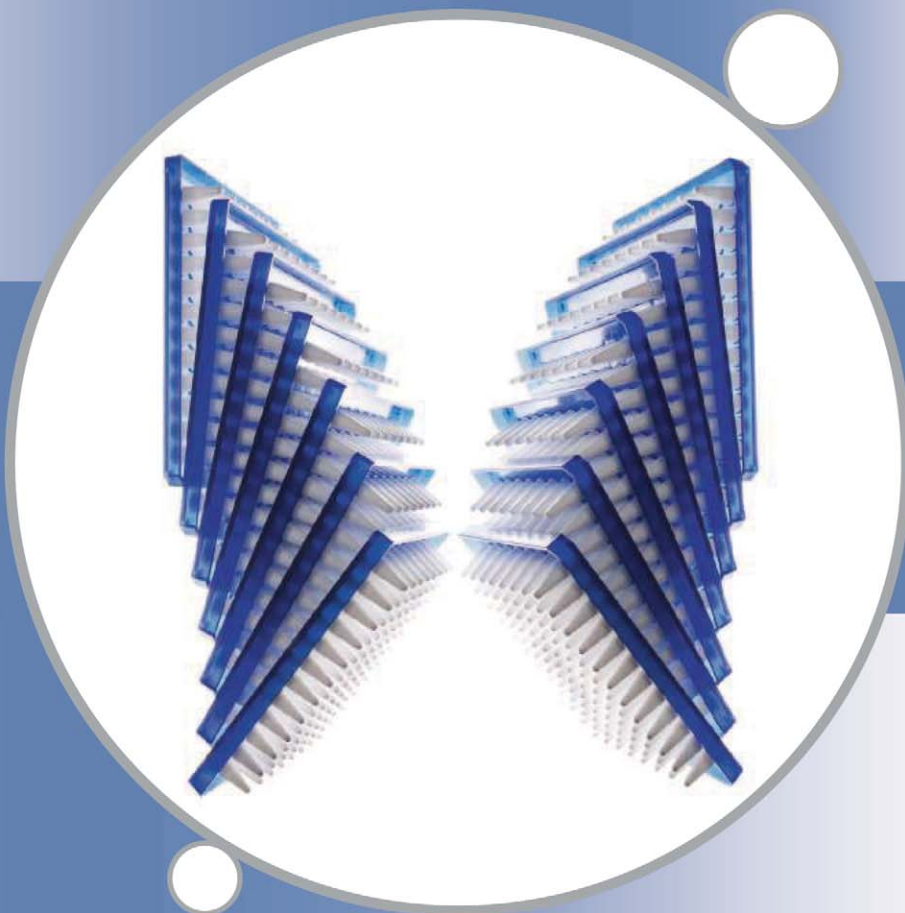
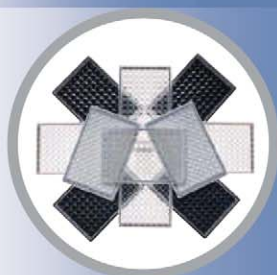
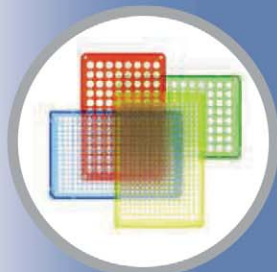
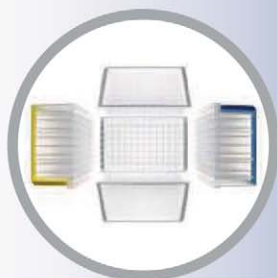


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Merged Cultures to Empower Women

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THE 55TH SESSION OF THE COMMISSION ON THE STATUS OF WOMEN (CSW) OF THE UNITED NATIONS (UN) closed last month with little fanfare, but there was cause for celebration. For the first time, the CSW, traditionally a forum to examine improvement of women's rights and gender equality, merged this broad interest with issues of science and technology (S&T). This makes sense because empowering women as scientists and engineers, supporting girls' education in science, and valuing women as builders of economic development all contribute to gender equality. The merger of these two areas produced recommendations to governments and nongovernmental entities that go far beyond what either group would likely have developed independently.

The Agreed Conclusions of the CSW member states* recognize the importance of S&T in every sphere of human existence, from combating malnutrition, the spread of disease, and environmental degradation, to building a more peaceful, secure, and prosperous world. Moreover, they acknowledge gender disparities in S&T at every educational level and in every sector of employment. The Agreed Conclusions emphasize how the use of S&T in poorer nations can free time for women to attend school or generate income. They also value women's indigenous knowledge in agriculture, health, and other sectors. The CSW member states call on governments, the private sector, and nongovernmental organizations to consider how both genders—and all ages—are affected differently by initiatives; to promote transparent and objective policies to recruit and retain women in science at all levels; and to create safe and gender stereotype-free school environments, thereby supporting girls who are interested in science, engineering, and math. They recognize the important role of media in cultivating societal attitudes as well as girls' and women's self-images; these attitudes can affect future career choices. The Agreed Conclusions are forward-looking and create a platform for action.

If the Agreed Conclusions are to be more than an idle document, then governments and nongovernmental stakeholders must take concerted action. Gender experts should boost efforts to develop and monitor S&T as well as innovation policies, programs, and practices. Funding entities must require analyses of the gender impact of projects, such as the development of new therapeutics or emergency preparedness measures. Science and engineering communities everywhere will need to draw upon the knowledge, language, and cultural ties of women scientists and engineers in diaspora communities—working with community-based groups to share knowledge and develop strategies that empower women in the various roles they play in their families, communities, and countries. As one example, the African Women in Agricultural Research and Development program (supported by the United States Agency for International Development, the Bill and Melinda Gates Foundation, and the Consultative Group on International Agricultural Research) strengthens skills of African women agricultural scientists, helping them to work effectively with many groups, including policy-makers and women farmers. UN bodies—including the recently launched UN Women, led by former Chilean President Michelle Bachelet, the UN Commission on Science and Technology for Development, and the United Nations Educational, Scientific and Cultural Organization—will be critical partners in moving this global agenda forward. And women's rights groups and science organizations will need to collaborate in creating a dialogue that builds on each others' strengths, advancing an agenda of common interests to drive meaningful action and change.

Women's rights, empowerment, economic development, and scientific talents are inextricably linked to the development of each nation. Only when all countries provide women with the tools they need to be equal partners will all nations flourish as part of a global community sharing a fragile planet.

— Kerri-Ann Jones, Shirley Malcom, Sharon Hrynkow†



10.1126/science.1206223

*www.un.org/womenwatch/daw/csw/55sess.htm †The authors were members of the U.S. delegation to the 55th session of the UN CSW. Other delegates are listed at www.state.gov/r/pa/prs/ps/2011/02/156814.htm.

BIOCHEMISTRY

Enzymes Aren't Perfect

Cells have efficient quality-control systems to detect and repair errors in polymerization of DNA or RNA. Tagliabracci *et al.* reveal the biological effects of errors by another polymerase, glycogen synthase, which creates glycogen—branched chains of glucose that serve as an important energy store in cells. About 1 in 1000 of the glucose residues in normal glycogen contain covalently linked phosphate. The authors found that the major enzymatic activity from mouse muscle catalyzing such incorporation of phosphate was in fact glycogen synthase. Although the incorporated phosphate may serve a useful function, current evidence indicates it is probably a mistake. A phosphatase that removes phosphate from glycogen is known as laforin because mutations in the enzyme are associated with Lafora disease, a deadly human disease that causes neurodegeneration and epilepsy. The disease appears to result from insolubility of excessively phosphorylated glycogen. Thus, laforin is likely required to prevent deleterious effects of catalytic errors made by glycogen synthase. — LBR

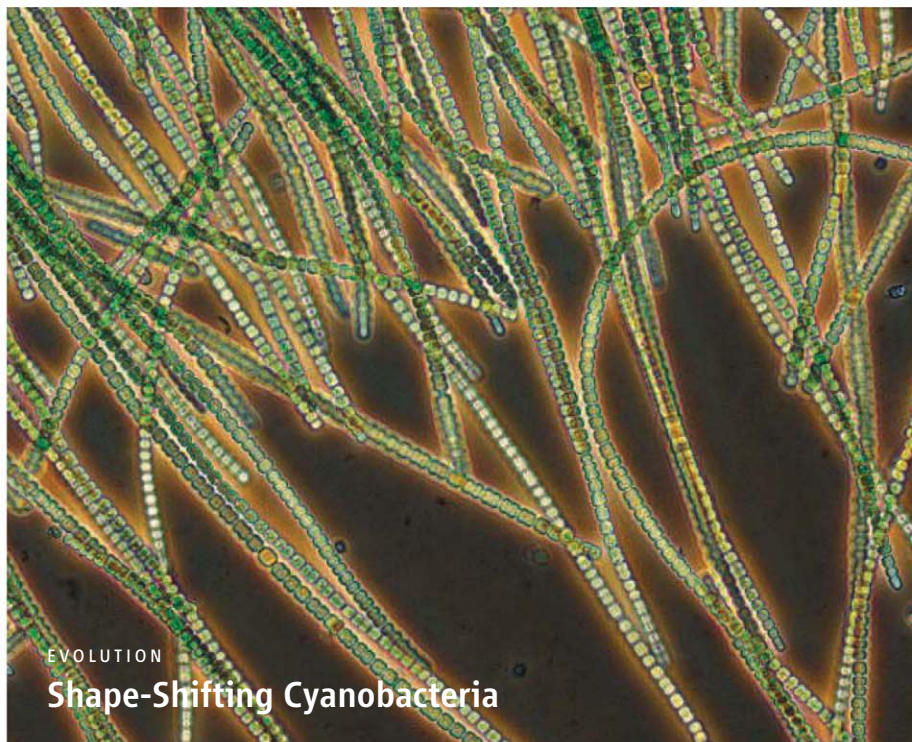
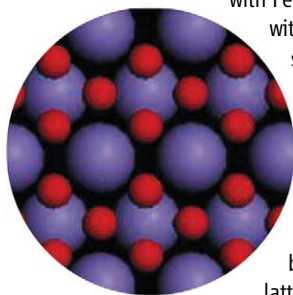
Cell Metab. **13**, 274 (2011).

MATERIALS SCIENCE

Flat Pack

Superlattices combining colloidal particles or nanocrystals with two distinct sizes can exhibit unusual magnetic and electronic properties. It has proven easier to make three-dimensional rather than two-dimensional structures of this sort, particularly for samples that extend over large areas. Dong *et al.* used a liquid-air fabrication method with a low concentration of nanocrystals in the feedstock to form single and binary superlattices that extend over cm² areas. The nanocrystals were deposited from hexane onto the surface of diethylene glycol and allowed to dry. Cocrystallization of Fe₃O₄ with Au, Fe₃O₄

with FePt, and NaYF₄:Yb/Er with Fe₃O₄ nanocrystals showcased the generality of the method. The superlattice structure could be controlled by changing the ratio of the diameter of the particles, with both AB- and AB₂-type lattices obtained. In hexagonally packed AB₂ lattices, the smaller particles occupied the interstices of three larger neighboring particles. Shifting to A₂B₃ bilayer structures (above), the authors found



EVOLUTION

Shape-Shifting Cyanobacteria

Two-and-a-half billion years ago, cyanobacteria may have caused the first mass extinction on Earth by inventing oxygenic photosynthesis. During their slow evolution, these prokaryotes donated their light-harvesting organelles to plants, developed a nitrogen-harvesting mechanism, and, not least, adopted many multicellular morphotypes. Few cyanobacteria have been sequenced, however, and as a result, their evolutionary history and phylogeny are largely unknown. Schirmeister *et al.* used multiple phylogenetic analyses on the limited number of 16S rDNA cyanobacterial sequences available to compare the resulting trees with established morphologically derived clades and to the scant fossil record. They obtained a monophyletic tree with *Gloeobacter* being the nearest outgroup and *Chlamydia* as the closest eubacterium. Rates of evolution among the cyanobacteria may be slow, but they organized into multicellular forms that displayed some functional specialization several times—and well before eukaryotes undertook this major transition. Since then, cyanobacteria appear merely to have evolved into three major clades, which have shifted between multicellular and unicellular morphotypes. Most modern lineages appear to have multicellular common ancestors, including the important marine genera *Synechococcus* and *Prochlorococcus*. — CA

BMC Evol. Biol. **11**, 45 (2011).

that the smaller particles sat on only half of the larger particles, and this anisotropy drove the formation of a beltlike morphology rather than a membrane. The method was extended to ternary ABC₂ structures using a mixture of three types of particles, although in this case the structure was more prone to defects. — MSL

Nano Lett. **11**, 10.1021/nl200468p (2011).

CELL BIOLOGY

Have miRNA, Will Travel

MicroRNAs (miRNAs), small noncoding RNAs encoded in the genomes of many eukaryotes, have a pervasive role in regulating gene expression.

miRNAs normally act cell-autonomously, but increasing evidence suggests that they might also act far from their site of synthesis.

High-density lipoprotein (HDL) particles facilitate the transport of lipids, and other biomolecules, in the bloodstream. Given that lipid-based transport of RNAs can be used as a systemic delivery system, Vickers *et al.* investigated the nucleic acid component of HDL and found that they are able to carry miRNAs. The profile of these miRNAs differed between healthy individuals and individuals suffering from familial hypercholesterolemia (which can lead to atherosclerosis), an effect also seen in a mouse model for atherosclerosis.

HDL loaded with exogenous miRNAs was able to modulate the expression of specific miRNA target genes when added to tissue-culture cells. Indeed, the changes in gene expression in cells exposed to HDL from individuals with familial hypercholesterolemia were enriched for genes involved in lipid metabolism, inflammation, and atherosclerosis, which suggests that some of the HDL miRNAs may play a direct role in disease progression. — GR

Nat. Cell Biol. **13**, 10.1038/ncb2210 (2011).

POLICY

Framing the Climate Debate

How concerned are Americans on the whole about global warming? Yeager *et al.* have found that the answer may depend on what exactly the question is. Pollsters regularly pose a “most important problem” (MIP) question originally devised by George Gallup in the 1930s: “What do you think is the most important problem facing the country today?” Only 1 to 2% of respondents to this question in three surveys conducted by the authors offered global warming or the environment as an answer. However, if instead the question posed was “What do you think will be the most serious problem facing the world in the future if nothing is done to stop it?” global warming/environment emerged as the most frequent response, its percentage more than 10-fold higher than before. Shuldt *et al.* examined the partisan subtleties of wording choice. They found that the Web sites of conservative think tanks use the phrase “global warming” more frequently than “climate change,” whereas the reverse was true of liberal think tank sites. They then surveyed a sample of Americans to probe the impact of these distinct phrases and found that self-identified Republicans were more likely (by a ~3:2 margin) to consider climate change a real phenomenon than global warming. Democrats were not affected by the wording, nor did educational attainment appear to favor one response over the other. — BJ

Public Opin. Q. **75**, 125;115 (2011).

MATERIALS SCIENCE

The (S)lowdown on Crystallization

One way to resolve the many steps that occur during crystallization is to use small droplets of solution, in part to avoid heterogeneous nucleation by impurities. Stephens *et al.* created picoliter droplets by using self-assembled monolayers (SAMs) that contained hydrophilic (carboxylic acid terminated) islands inside hydrophobic

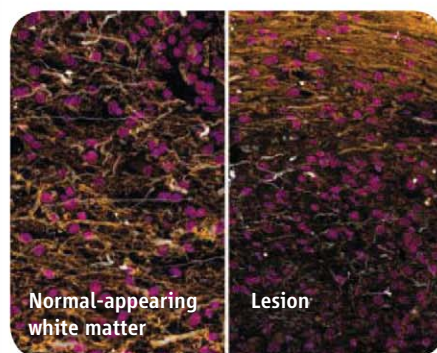
(fluorous terminated) layers. These islands supported hemispherical droplets, with radii varying from 4 to 10 μm (0.04 to 2 pl in volume), that were supersaturated with calcium carbonate. Bulk precipitation created many rhombohedral crystals, but in 90% of the droplets, a single smaller tetrahedral crystal formed. Redissolution of these tetrahedral crystals in undersaturated solution, or further growth from bulk solution, initiated recrystallization into rhombohedral crystals. The SAMs appear to slow down crystallization and capture the tetrahedron as an early intermediate crystal form. — PDS

J. Am. Chem. Soc. **133**, 10.1021/ja200309m (2011).

BIOMEDICINE

Axon Damage Illuminated

Pathogenesis of the autoimmune disease multiple sclerosis (MS) is associated with progressive deterioration of the myelin sheath surrounding neuronal axons; however, axon damage may also contribute to MS-associated neurodegeneration. Nikić *et al.* used in vivo imaging and electron microscopy to examine axon damage in a mouse model of MS (EAE, experimental autoimmune encephalitis). In EAE mice, swelling in discrete sites on axons was observed, which was then followed by axon fragmentation. In many cases, damaged axons retained myelin; in



some cases, axon damage was reversible. Axon damage was preceded by mitochondrial pathology, which was associated with the presence of microglia and the production of reactive oxygen and nitrogen species. Induction of oxidative or nitrosative stress was sufficient to induce mitochondrial pathology and axon damage in normal mice, and their blockade in EAE mice alleviated axon damage. Lesion biopsies from MS patients also showed similar axon (above, right) and mitochondrial damage, which suggests that reversing axon damage may be an important therapeutic strategy in the treatment of MS. — KLM

Nat. Med. **17**, 10.1038/nm.2324 (2011).



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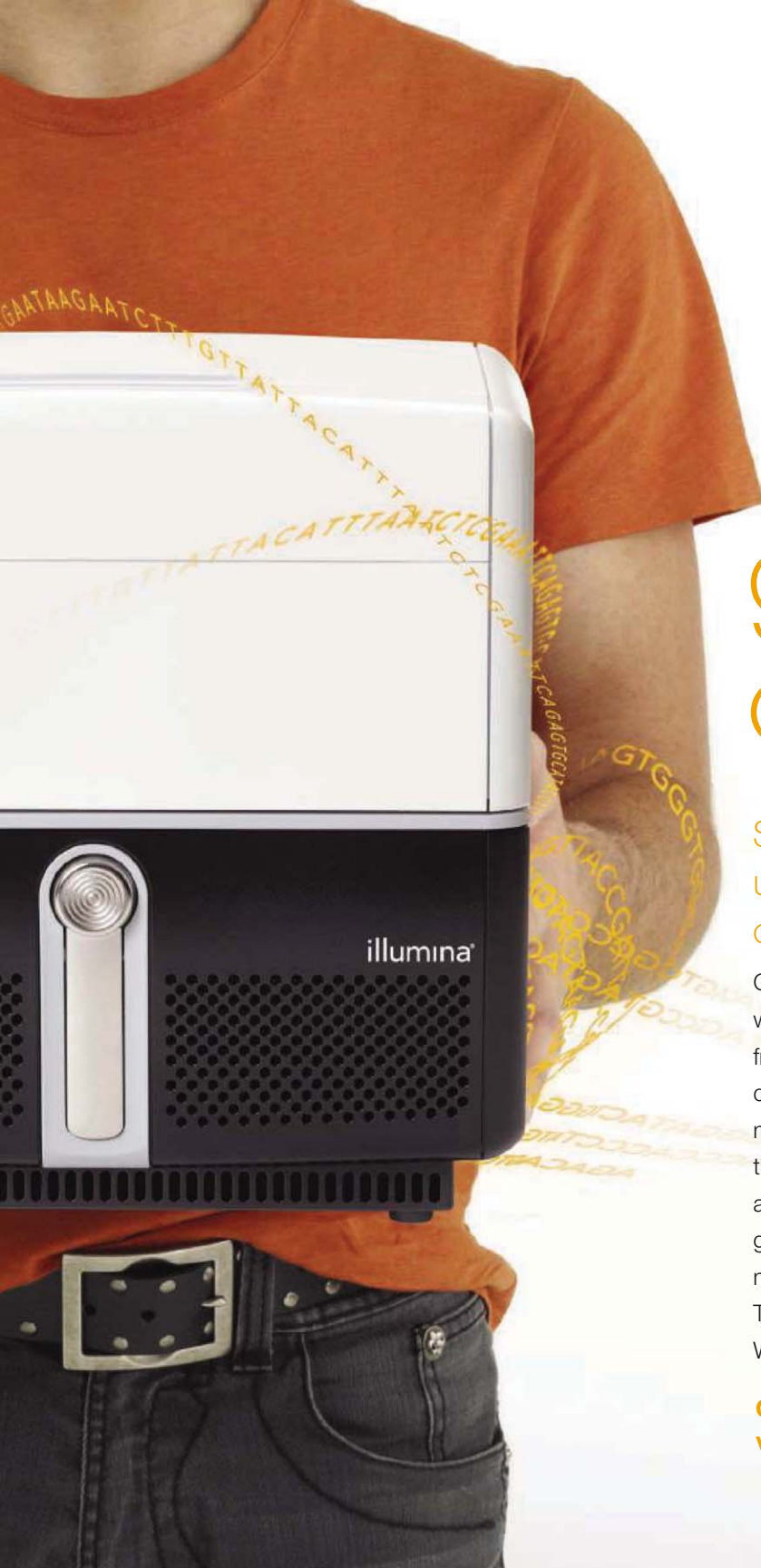
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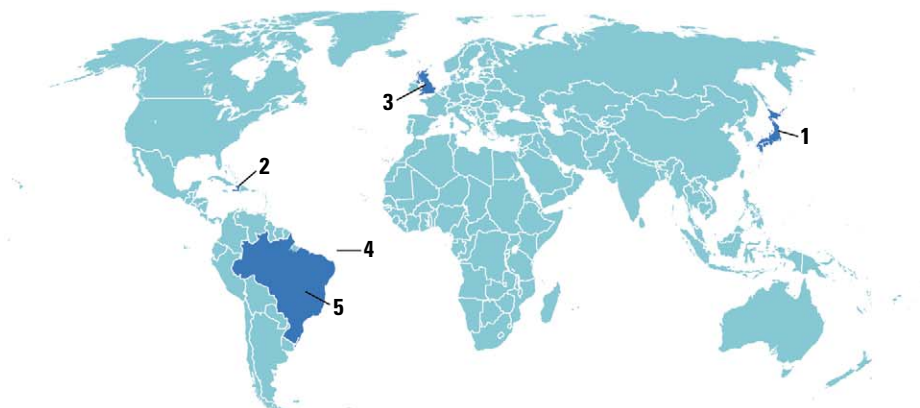
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AROUND THE WORLD



Fukushima, Japan 1

Nuclear Crisis Drags On

As *Science* went to press, engineers were trying to staunch the flow of highly radioactive water from the devastated Fukushima Daiichi Nuclear Power Plant. Alarming concentrations of radioactive iodine-131 and cesium-137 have been detected around the plant and in nearby seawater. Authorities suspect that water pumped in to cool the unit two reactor is leaking into a cracked concrete utility pit, from which it is seeping into the ground and then into the ocean. “We have to do everything we can to stop the leakage as soon as possible,” Noriyuki Shikata, a government spokesperson, said at a press conference on 4 April. In addition, there will be an intentional release of 11,500 tons of slightly radioactive water from a holding tank into the sea to make room to store more dangerously contaminated water.

The 11 March tsunami knocked out fishing fleets in the area near the power plant. Japan’s Fisheries Agency says it has detected no significant radiation in catches from neighboring areas so far. Meanwhile, plant workers have been trying to restore cooling systems to stabilize the nuclear fuel in the reactor cores and spent fuel pools.

Haiti 2

New Studies Support Asian Source of Cholera

Two recently published studies support the theory that Haiti’s cholera outbreak was introduced from South Asia—but neither specifically fingers the U.N. peacekeeping forces from Nepal that some suspect inadvertently brought the disease to the island last October (*Science*, 28 January, p. 388).

The two genetic analyses—one pub-

lished in the April issue of *The Lancet Infectious Diseases*, the other online 18 March in *Emerging Infectious Diseases*—both found that Haiti’s strain most closely resembles cholera samples collected in South Asia, providing “independent corroboration” that the microbes were imported from that part of the world, says Matthew Waldor of Harvard Medical School in Boston, who drew the same conclusion in a January paper in *The New England Journal of Medicine*. To prove



a Nepalese connection, however, researchers would need to compare Haitian microbes with a much larger set of samples from various Asian countries, he adds. Waldor and other cholera scientists are eagerly awaiting the report by a U.N. expert panel on the outbreak, slated for release within 3 weeks.

Daresbury, U.K. 3

Scientists Take New Accelerator on Test Run

A new type of particle accelerator, which promises to provide particle beams more cheaply and efficiently than existing types for applications ranging from cancer treatment to nuclear power, passed a major milestone last week when it circulated a beam all the way around its circumference and accelerated it to 18 megaelectron volts.

The accelerator, known as EMMA (Electron Machine with Many Applications) and

built at the Daresbury Laboratory in the United Kingdom, is the world’s

first nonscaling fixed-field alternating-gradient accelerator, or nonscaling FFAG. Such machines don’t have the accelerating power of CERN’s Large Hadron Collider. But their use of smaller, simpler magnets allows them to produce particle beams that are more affordable for applications such as proton beam cancer therapy, scanning cargo for explosives, and inherently safe nuclear reactors known as accelerator-driven systems.

“Now that we know that the basic idea works, we can go on to develop the applications of this new technology,” says team member Ken Peach, director of the John Adams Institute for Accelerator Science at the University of Oxford and Royal Holloway University of London.

South Atlantic Ocean 4

Oceanographers Find Failed Flight

Only days into the fourth search for an ill-fated 2009 flight, oceanographers have found the remains of Air France Flight 447 3900 meters deep in the Atlantic, about 500 kilometers off northeast Brazil. The three autonomous underwater vehicles (AUVs), operated by oceanographers from Woods Hole Oceanographic Institution in Massachusetts, first located debris on relatively smooth seafloor using sonar; an AUV then returned to take photographs, which provided definite identification. The wreck isn’t far from the spot that French authorities had eventually identified as the most likely resting place for the crash. A follow-up investigation using a remotely operated vehicle able to grapple objects will attempt to recover the plane’s flight recorders.

Brasília, Brazil 5

Alleged Fraud Spurs New Scientific Integrity Commission

Brazil’s science ministry will create a commission on scientific integrity after Elsevier said it would retract 11 papers, the senior author of which is Claudio Airoidi, 68, a chemist at the State University of Campinas, known as Unicamp.

Elsevier launched an investigation after receiving complaints about the work; a review by three independent experts concluded the papers contained forged nuclear magnetic resonance (NMR) spectra. Both



Airoidi, who has co-authored over 400 papers, and Denis Guerra, the first author on the retracted papers, denied the accusations. Guerra, now at the Federal University of Mato Grosso, was quoted in the Brazilian press as saying, “There is no need for a Unicamp researcher to fabricate data. It’s an absurd accusation.”

Although the retraction of the thinly cited papers is unlikely to have much scientific impact, it could change the way fraud allegations are handled in Brazil. Brazil’s key funding agency, the National Council for Scientific and Technology Development, has so far left fraud probes to universities. Now, officials in Brasilia say they will create their own investigative body. Airoidi, who holds the agency’s highest ranking, was suspended from reviewing federal grants until investigations are complete.

NEWSMAKERS

Three Q’s

Caltech neuroscientist **Christof Koch**, famous for studying the biological nature of consciousness, is moving from Pasadena to Seattle to become chief scientific officer of the Allen Institute for Brain Science. The institute is best known for creating atlases of gene expression in the brain.



Q: Does this mark a turning point for the Allen Institute?

Until now, they’ve focused on these atlases, which you can think of as static information. Now what we want to do is much more dynamic, we want to record the electrical activity of large numbers of neurons in the brain during certain well-specified behaviors, using a range of techniques. The mission is to understand how information is encoded and processed in the brain.

Q: How will you decide which questions to ask with this technique?

Delicately. Nobody has done this kind of high-throughput electrophysiology. Some people will say it can’t be done, but I don’t think that’s true. The difficulty is picking goals and milestones that are achievable in the next 10 years.

Q: What’s one question you’re considering?

One example is 40 hertz gamma oscillations [of electrical activity in the brain]. They were discovered a long time ago, but in 2011 if you ask a bunch of neuroscientists what they do or are they even there, you get totally different answers. Why? Because there are no unified standards for experiments like in other areas of science. People are more likely to use each other’s toothbrush than to use each other’s protocols. So even for basic questions like this, we still don’t have an answer.

Astrophysicist Honored For Asking ‘Vital Questions’

The Templeton Foundation has awarded its annual prize to astrophysicist **Martin Rees** for his “profound insights on the cosmos [that] have provoked vital questions that speak to humanity’s highest hopes and worst fears.” Currently Master of Trinity College at the University of Cambridge in the United Kingdom, Rees has a seat in the House of Lords and served as president of the Royal Society from 2005 until November 2010. He has written numerous books and papers on the philosophical questions raised by the physics of the universe’s beginnings, as well as how human activities will determine the Earth’s future.



The \$1.6 million prize is the largest award for an individual, and honors a person who has “made exceptional contributions to affirming life’s spiritual dimension.” “I hadn’t thought I had the basic entry qualifications, looking at the previous winners,” Rees says. “I’m proud to be joining that roll call.”

The foundation’s mission has concerned some scientists, who have accused it of blurring religious and scientific values. Unlike many past awardees, Rees is not religious but says he is “inspired” by religion’s contributions to the arts and feels that philosophy and ethics are important to scientific inquiry.

Really Big Dinosaurs Take Over New York

The biggest animals ever to walk the Earth are about to invade the Big Apple. From 16 April to 2 January 2012, the American Museum of Natural History (AMNH) in New York City is hosting an exhibit devoted to “The World’s Largest Dinosaurs,” the gargantuan, plant-eating sauropods that roamed the planet 200 million to 65 million years ago. The show’s centerpiece is a life-size model of *Mamenchisaurus*, who weighed 20 metric tons and whose 9-meter-long neck made up half of its body length. (Some of its sauropod cousins were up to four times heavier.) Parts of the model will be cut away

to reveal its skeleton, muscles, heart, and digestive system.

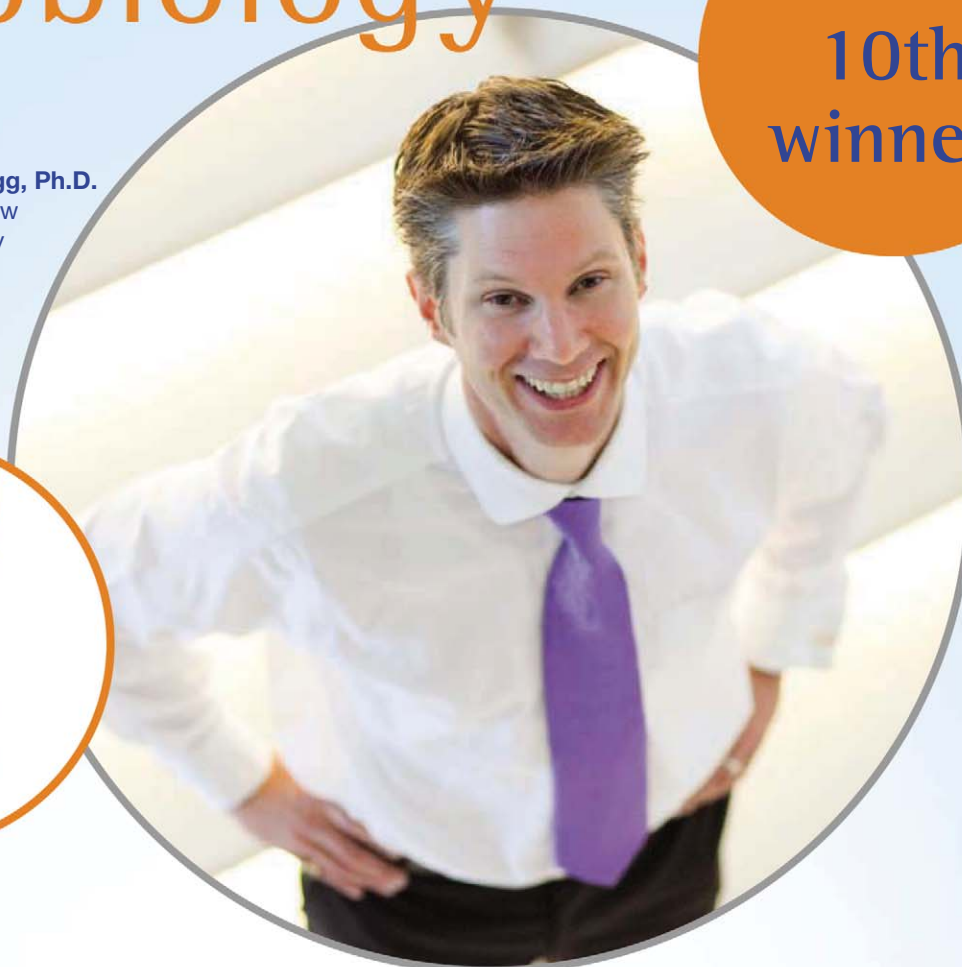
These dinosaurs may be ancient, but the science will be new. AMNH paleontologist Mark Norell, curator of the dino show, says that the exhibit will focus on recent findings by paleontologist Martin Sander of the University of Bonn in Germany, whose team has been studying why dinosaurs got so huge in the first place (*Science*, 10 October 2008, p. 200). “This exhibit is about ... the evolutionary solutions they developed to cope with their enormous size,” Norell says.



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Congratulations to Dr. Christopher Gregg on winning the 2010 Eppendorf & Science Prize for his studies on genes that alter their expression in the brains of offspring according to whether they were inherited from the father versus the mother. His findings suggest new pathways that may help to understand brain diseases such as autism, schizophrenia and eating disorders.

The annual international US\$ 25,000 Eppendorf & Science Prize for Neurobiology honors young scientists for their outstanding contributions to neurobiological research based on methods of molecular and cell biology. The winner and finalists are selected by a committee of independent scientists, chaired by *Science's* Senior Editor, Dr. Peter Stern.

To be eligible, you must be 35 years of age or younger. If you're selected as this year's winner, you will receive US\$ 25,000, have your work published in *Science* and be invited to visit Eppendorf in Hamburg, Germany. Past winners and finalists have come from as far a field as China, Chile, India and New Zealand. Yes, it *can* happen to you!

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BY THE NUMBERS

1.21 billion India's population, according to last week's census results. That's up 181 million since 2001, the equivalent of moving nine out of every 10 Brazilians to India.

9.57 Amount, in zettabytes (10^{21} bytes), of business-related information processed by the world's computer servers in 2008, according to a report by scientists at the University of California, San Diego.

FINDINGS



The Curse of the Mummy's Arteries

In the ancient tomb paintings of the Nile Valley, Egypt's nobility often appear lithe, beautiful, and healthy. But new research, the latest being a study presented this week at the American College of Cardiology's annual scientific session, paints a less wholesome picture of ancient Egyptian health.

A team led by cardiologists Adel Allam of the Al Azhar Medical School in Cairo and Gregory Thomas of the University of California, Irvine, performed CT scans on 52 mummies. Of the 44 who still possessed identifiable cardiovascular tissue, 45% exhibited definite or probable atherosclerosis, or hardening of the arteries. The average age of death was 40.

How did Egypt's upper echelon get so unhealthy? Wealthy ancient Egyptians relished such calorie-rich fare as cakes sweetened with honey, but they did not smoke tobacco and, in an age before automobiles, they likely got more exercise than many of us do today. So the researchers think that other risks, such as high exposure to pathogens—malaria, for instance, is endemic to the Nile Valley—came into play. Chronic infections cause high levels of inflammation, which can contribute to the hardening of arteries.

Random Sample

Death of a Polar Bear

A new scanning-and-prototyping technique has helped to explain why Knut, the world-famous polar bear, died suddenly in front of hundreds of shocked visitors to the Berlin Zoological Garden last month. Thomas Hildebrandt and colleagues at the Leibniz Institute for Zoo and Wildlife Research (IZW) in Berlin used their bear-sized computed tomography scanner (*Science*, 16 July 2010, p. 261) to scan Knut's body. Then, with Ben Jastram and Joachim Weinhold of the Technische Universität Berlin's 3D Lab, they made hyperaccurate replicas of his skull, brain, and face (pictured). The necropsy conducted by pathologist Claudia Szentiks at IZW identified encephalitis, possibly viral, as the cause of Knut's apparent seizure and fall into the water in his enclosure, where he drowned. The scans and models showed that the brain was structurally normal, ruling out an inborn defect and explaining why Knut showed no symptoms before his deadly fall. "It helped to show that the zoo couldn't have known he was sick," Hildebrandt says. Polar bears in the wild sometimes fight to the death and so have an evolutionary reason to hide any symptoms of disease or other weakness, he says. The researchers are also using data from the scans to create a virtual tour of Knut's insides.



The team now plans to test this hypothesis by examining CT scans of the mummies for signs of chronic infections.

<http://scim.ag/mummy-curse>

Chinese Ducks Felled By New Virus

Peking duck, salted duck eggs, duck soup: China is famous for its duck delicacies and duck farms dot the country's agricultural belt. So last spring, when egg production plummeted by as much as 90% in some flocks, Chinese farmers began to worry.

Some ducks died within days of falling ill. By the end of the year, an estimated 4.4 million ducks in eastern China had caught the mysterious illness, which then spread farther afield.

When the news reached microbiologist George Gao and his colleagues at the Chinese Academy of Sciences in Beijing, they headed to the farms to collect tissue and serum samples from the affected flocks. As detailed in a paper published 24 March in *PLoS ONE*, they isolated the culprit: an aggressive new flavivirus, a class of viruses that includes yellow and dengue fevers. Dubbed BYD, it is the first flavivirus ever identified in ducks. Gao and colleagues stress that the disease should be closely monitored; many flaviviruses can be transmitted from animals to people. The next step, they say, is the development of a BYD vaccine.

<http://scim.ag/duck-virus>

Paper, Plastic, or Steel?

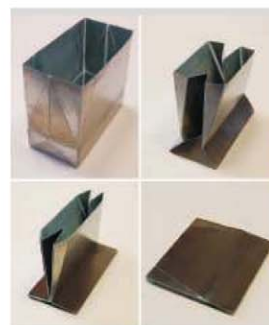
Inspired by the ancient art of origami, engineers have built a foldable grocery bag from steel (go ahead, load it with soda bottles!). The technique could help speed up factory packaging processes.

By adding a number of extra creases to the traditional pattern used in conventional paper grocery bags, Zhong You and Weina Wu of the University of Oxford in the United Kingdom constructed a prototype of a steel bag that can be collapsed as flat as a standard paper grocery bag (pictured). The bag is made of stainless steel plates stuck to a light, flexible plastic sheet.

The edges where the plates meet serve as "creases," along which the bag can be bent.

The prototype could lead to what was previously thought impossible to make: cardboard boxes that can be flat-packed while keeping their bottoms intact, thus saving time on factory assembly lines. The pair are now discussing their design—published online last week in the *Proceedings of the Royal Society A*—with carton manufacturers.

<http://scim.ag/steel-bag>



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Getting tested. A health care worker draws blood from a boy in Cairo to test for HCV, which infects more than 10% of Egyptians.

INFECTIOUS DISEASES

First Specific Drugs Raise Hopes for Hepatitis C

BERLIN—A virus that chronically infects 170 million people around the world is about to meet some new and formidable foes. Regulatory agencies in both the United States and Europe are soon expected to green-light the first two antiviral drugs specifically developed to treat chronic hepatitis C, a treacherous infection that can cause cirrhosis of the liver and liver cancer, often decades after infection occurs.

The drugs, both of which target a viral enzyme called the NS3-4A protease, promise to rid 70% to 80% of patients of their infection—a significant step up from the current, nonspecific therapy, which cures barely half of those treated. And they are just the first to emerge from a pipeline bursting with other candidates, which has galvanized the field and made hepatitis C the most prominent topic at the International Liver Congress held here last week. “This is a disease we should be able to lick,” says Charles Rice of the Center for the Study of Hepatitis C at Rockefeller University in New York City.

Yet Rice and others caution that there is a lot of work ahead and that the two new protease inhibitors have significant problems—including side effects, high costs of perhaps

as much as \$20,000 per patient, and resistance in the ever-changing hepatitis C virus (HCV). Their introduction later this year could create “a hangover after the party,” noted Heiner Wedemeyer, secretary general of the European Association for the Study of the Liver in Geneva, Switzerland.

Many drug companies have entered the hepatitis C field because of the large number of infected people. But they have to hurry. Most patients were infected through blood transfusions before the virus was discovered in 1989. Since then, screening has made transmission through donated blood rare, and although other modes of transmission exist—such as needle sharing by injecting drug users and some sexual

practices—infection rates in Western countries have dropped sharply. As more patients are treated or die, the market in rich countries will start shriveling. “The companies are all racing against each other, and against time,” says Kenny Simmen, the vice president for virology research at Janssen, the Belgian company that has developed one of the new protease inhibitors, telaprevir, together with Vertex in Cambridge, Massachusetts. (The other one is Merck’s boceprevir.)

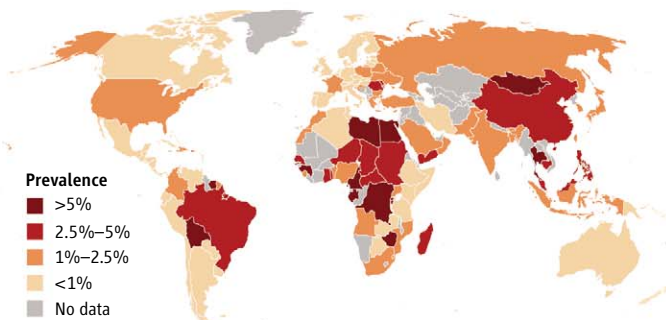
The current standard therapy is a combination of pegylated interferon α , a compound that has a wide range of virus-fighting effects on the immune system, and ribavirin, an antiviral that does little against HCV by itself but boosts interferon α ’s effects. That combination can cause flulike symptoms and depression and needs to be taken for at least 24 and often 48 weeks; yet it completely eliminates the virus in just 40% to 50% of patients.

In phase III studies that added the new protease inhibitors to the standard mix, the cure rate shot up to 70% to 80%, even for genotype 1, the most difficult-to-treat type of HCV and the most common in the United States, Europe, and Japan. But the new drugs also add their share of side effects: Telaprevir can cause anal itching and rash, and boceprevir can cause a bad taste in the mouth; both cause anemia.

Indeed, the current strategy is so “scary” that doctors may have trouble persuading some patients who have tried it in vain to give it another try with a protease inhibitor added in, Rice says. Other questions remain as well. Hepatologists would like to see much more data on safety and efficacy in patients who are already suffering from cirrhosis, for instance.

The ultimate goal for the field is to ditch ribavirin and interferon α through a combination of two, three, or four HCV-specific antiviral drugs, says Johan Neyts of the Rega Institute for Medical Research in Leuven, Belgium. A triple or quadruple whammy might prevent the emergence of resistance seen in patients treated with one or two specific antivirals, he says.

Plenty of antiviral candidates are in phase II and III trials, aimed at a variety of targets. More protease inhibitors are coming, but so are drugs targeting other viral proteins, such as an enzyme called poly-



A hidden problem. WHO estimates that 170 million people are chronically infected with hepatitis C, most of them without knowing it.

merase. Promising preliminary results from a combination of two polymerase inhibitors, both produced by a company called Pharmas-set, made its stock price soar in early March.

Other companies are targeting human proteins, such as Cyclophilin A, which is essential in HCV's replication; promising phase II results presented here showed that one of these drugs, Novartis's alisporivir, is much harder for the virus to elude than most other candidates. Eventually, a series of clinical trials will likely establish one or more combinations with a high cure rate, a short duration, and few side effects, says Neyts—but it may take the best part of the decade.

Meanwhile, the advent of better treatments has helped propel hepatitis C onto countries' political agendas and triggered a push for awareness. Even in Western countries, many infected people—about a third in the United States—don't know their status. "There is no other disease in which the

gap between the scale of the problem and the level of awareness is so wide," says Charles Gore, president of the World Hepatitis Alliance, a patient group. Many countries plan to expand screening among risk groups and encourage patients to get treatment; the U.S. Health and Human Services Department, for instance, will unveil a new action plan next month.

The challenges are greatest in the developing world, where most patients reside. In Egypt, more than 10% of the population is infected with hepatitis C, the result of 1970s and '80s treatment campaigns against schistosomiasis, a parasitic disease, in which needles were often reused. Transmission through unsafe medical practices continues even today, says Arnaud Fontanet, an epidemiologist at the Pasteur Institute.

The astronomical price of treatment is a huge obstacle. In Egypt, negotiations have brought the price of interferon α and riba-

virin down by about 90%, to some \$3000 per patient, but that is still far too high, Fontanet says. The new wave of drugs will make treatment more expensive. China, which has 40 million patients—more than any other country—and a burgeoning biotech and pharmaceutical industry, might be better off developing its own cheap antiviral drugs, says Ralf Altmeyer, director of the Pasteur Institute in Shanghai.

Yet, "we have more immediate issues than price," says Steven Wiersma, who has set up a new unit for viral hepatitis at the World Health Organization in Geneva. The first order of business, he says, is to raise the profile of the disease, gather data on prevalence, and halt transmission. "We don't have basic awareness of what the disease is among the governments and the health professionals. ... We're several steps away from even discussing prices."

—MARTIN ENSERINK

ENVIRONMENT

First Detection of Ozone Hole Recovery Claimed

The feel-good environment story of recent decades—the recovery of the so-called ozone hole—may be passing a major milestone far ahead of schedule. Every austral spring, an ominous "hole" appears in stratospheric ozone over Antarctica. In 1989, international regulations began restricting emissions of ozone-destroying chemicals, such as chlorine-containing refrigerants and propellants. The restrictions quickly capped and then began steadily reducing atmospheric concentrations of those dangerous chemicals.

Although ozone-destroying chemicals have been in decline for a decade now, researchers have long projected that they will not glimpse the first signs that the hole is healing until well past 2020. But for the first time, a group of researchers claims they can already see the ozone hole slowly recovering. Many others, however, say the paper, now in press in *Geophysical Research Letters* (GRL), leaves out critical information needed to clinch the case.

No one had been expecting to discern the recovery quite so soon. Scientists thought large, wholly natural, year-to-year variations in Antarctic ozone would obscure any small upward trend. Those natural ozone variations can be traced back through physical and

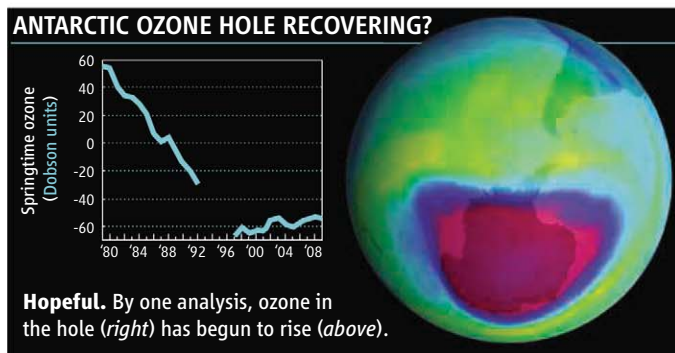
chemical changes in the atmosphere to natural changes in atmospheric circulation. But no one had been able to take those circulation changes into account accurately enough to reveal the underlying ozone recovery.

Meteorologist Murry Salby of Macquarie University in Sydney, Australia, and his Australian colleagues think they have nailed down

Subtracting their estimate of the natural changes in ozone from actual changes, the group finds "a clear upward trend since the late 1990s" in the hole's ozone that represents "a systematic rebound." Over the past decade, the rebound has amounted to about 15%, they estimate. "It's a small trend, but it's all we've been waiting for," says meteorologist Lorenzo Polvani of Columbia University. "Now we're actually seeing it."

Some other researchers aren't so sure. "I'm cautiously optimistic," says ozone researcher Paul Newman of NASA's Goddard Space Flight Center in Greenbelt, Maryland, but "I've done these sorts of correlations, and if you vary all the [uncertain parameters] and redo the analysis, you'll get different correlations. They won't look as good." Newman and others want to see a quantitative measure of the uncertainties.

The authors argue that the uncertainty is obviously small. They point to the way their estimate of chemically destroyed ozone closely tracks the amount of chemicals in the hole during the past 30 years. If they had chosen the wrong values, they say, those two trends would not have matched as well. Sorting out just what sort of uncertainty accounting is required should take at least until next October's hole shows up. —RICHARD A. KERR



Hopeful. By one analysis, ozone in the hole (right) has begun to rise (above).

the natural influences on the hole's ozone. In their *GRL* paper, they consider how much of the observed polar ozone variation each spring could be accounted for by changes in two kinds of atmospheric circulation that ultimately influence the amount of ozone destroyed in making the hole each spring. Taken together, the two observed that circulation changes accounted for "virtually all" of the year-to-year changes in springtime Antarctic ozone, Salby and colleagues write.

CHINA

Daring Experiment in Higher Education Opens Its Doors

SHENZHEN, CHINA—Let's say a pirate ship is 560 meters from a seaside fort. If the fort can fire a cannonball at a speed of 82 meters per second, at which two angles could you raise the cannon and hit the ship? David Shuk Yin Tong, teaching Physics 101 in English, poses that question to the inaugural batch of freshmen here at South University of Science and Technology of China (SUSTC). A dozen hands shoot in the air.

Now let's say the pirate ship is a new university that's taking aim at sacred cows of China's education system. So far the vessel has not been blown out of the water. But the stress is taking a toll on its commander, Zhu Qingshi. "I haven't been sleeping well for months," he confides. Last month, Zhu launched SUSTC, a bold challenge to the country's education system. Among Chinese universities, SUSTC stands alone in spurning the national entrance exam, or *Gao Kao*: it enrolls high-flying students nominated not only for their grades but also for their creativity and passion for learning. In another innovation, SUSTC faculty members are not given administrative rank. In China, Zhu says, many professors spend more energy climbing this bureaucratic ladder than improving their teaching. And whereas other mainland universities have two leaders—president and Communist Party secretary—Zhu holds both titles and calls all the shots.

The central government has qualms about SUSTC's revolutionary approach; the education ministry has not granted it accreditation. SUSTC "wants to operate in a totally new mode. That will be a great challenge," says Hao Zhifeng, a vice president at Guangdong University of Technology in Guangzhou.

Zhu, 65, had no illusions that the road would be easy. But if he can succeed anywhere, it's here. Thirty years ago, China made a momentous decision to experiment with a market economy in Shenzhen. "We need to do the same with our education system," Zhu says. Put another way, says SUSTC mathematics professor Zhang Xianke, "China's GDP has risen, but now science and education need to rise. We need to improve the way our people think." The fact that the Chinese government has allowed SUSTC to set sail "is an indication of progress" toward education reform, says Gerard Postiglione, director of the Wah Ching Center of Research on Educa-

tion in China at the University of Hong Kong.

Zhu himself is exceptional: The physical chemist's pioneering work in laser spectroscopy won him election to the Chinese Academy of Sciences (CAS) at the age of 45. As president of CAS's University of Science and Technology of China in Hefei from 1998 to 2008, Zhu had a bird's-eye view as his teaching staff and students struggled to transform bookish smarts into creative sparks. Many talents, he observed, blossomed only after they went overseas for advanced training. "If China wants to become a leader in higher education," Postiglione says, "more creative

ter science university than Hong Kong," Hao says. Shenzhen paid nearly \$1 billion for the land for SUSTC's campus. Construction will cost another \$300 million and is expected to be completed in June 2012. SUSTC plans to enroll 200 students next year. Its target in 5 years is 300 teaching staff, 2000 undergrads, and 100 graduate students.

The university opened its doors 2 weeks ago to 45 freshmen at a temporary campus. Even this elite group has an outlier: 11-year-old Su Liuyi, who has already managed to wow his mathematics professor. "Su is not only clever, he also likes thinking. I've already introduced him to group theory," says Zhang, who gave up a professorship at Tsinghua to come to SUSTC.

The students know that they have taken a gamble in enrolling. "We don't have enough faculty, students, or even books in our library" to qualify officially as a university, Zhu says. Even if SUSTC expands as planned, the law requires many years of operation "before we can enroll Ph.D. students," Zhu says. To overcome this hurdle, he must persuade the central government to either change the law or grant SUSTC status as an experimental post-graduate institution.

Zhu has nevertheless managed to reel in a few outstanding faculty members, including Zhang, Tong, who is a former deputy president of City University of Hong Kong, and supercomputer specialist Chen Guoliang. As an inducement for others to follow, Zhu is building a research endowment. "We want our professors to never have to worry about funding.

They can focus on research," he says.

The central government has given SUSTC 3 years to prove it deserves accreditation. If the university fails to make that case, its graduates may have little choice but to go abroad for further study or jobs; few companies in China would recognize their diplomas. Considering the sizable sum that Shenzhen is investing in SUSTC, Hao says, "no one wants to see it turn into a high-level prep school that sends elite Chinese students overseas."

Zhu understands: "If society does not accept our students, it will mean we have failed." On the other hand, if SUSTC prevails, he says, "it can be an example for the whole country."

—RICHARD STONE



Young pioneers. SUSTC's inaugural class has placed their fate in the hands of Zhu Qingshi (left), who has persuaded scholars like Zhang Xianke (right) to join his revolution.

and deep-thinking students will have to come from Chinese universities."

That will require universities to reinvent themselves. Powerhouses like Tsinghua University and Peking University, both in Beijing, are attempting to do that through gradual evolution. Another initiative announced last week will try a hybrid strategy: New York University is partnering with East China Normal University to create NYU Shanghai. Expected to open in September 2013, the research university will also look beyond *Gao Kao* scores during admissions and enroll up to half its students from outside China.

SUSTC is taking a more radical tack, with local support. Shenzhen officials "thought they could spend more money and build a bet-

SCIENCE EDUCATION

DOE Pulls the Plug on Massive Training Initiative

In April 2009, President Barack Obama announced an ambitious education and training initiative at the Department of Energy (DOE). Speaking to members of the U.S. National Academy of Sciences, Obama said a proposed 10-year, \$1.6 billion program, dubbed “Regaining our Energy Science and Engineering Edge” (RE-ENERGYSE), would “capture the imagination of young people who can help us meet the energy challenge.”

But RE-ENERGYSE never got off the ground. Congress twice declined to fund any portion of its sprawling vision, which would have included graduate and postdoctoral fellowships, summer research projects for undergraduates, professional master’s degrees in clean energy, and associate degree programs to train a clean-energy technology workforce. In February, the White House threw in the towel, dropping the program from DOE’s 2012 budget request.

Energy Secretary Steven Chu insists that the Administration remains gung ho about attracting more students into the field of clean-energy research. But its 2012 budget request signals a major shift. Instead of RE-ENERGYSE, Chu is now touting expansion of a small graduate fellowship program that is run out of an office different from the one responsible for implementing RE-ENERGYSE.

To understand why RE-ENERGYSE never got off the ground, it helps to under-

stand DOE’s checkered history in science education. Although some previous Administrations tried to carve out a larger role for DOE in this arena, Congress has traditionally seen education as a secondary mission for a department that already has major responsibilities for science, energy, national security, and environmental cleanup. So the broad scope of RE-ENERGYSE was a red



Out of energy. President Obama’s 2009 speech to the National Academy of Sciences touted a program that never happened.

flag for some influential legislators, who also wondered why it was under the jurisdiction of the energy undersecretary when existing education and workforce-training efforts were overseen by the science undersecretary (*Science*, 10 July 2009, p. 130).

Kristina Johnson, the former energy undersecretary whom Chu asked to manage RE-ENERGYSE, says that it seemed clear

to her. “It’s a national imperative, and the president is strongly behind it,” she explains. “So I came in, very wide-eyed, and said we should do this. As an engineer, I like to set a goal and put in place a plan and then carry it out. It’s really pretty simple.”

Although Chu tried to explain the program to legislators when testifying on DOE’s 2010 budget, Congress eliminated his request for \$115 million when it approved DOE’s overall budget. The next year’s request, for \$55 million, fared no better. And last October, Johnson, a former engineering dean and successful entrepreneur who joined the Administration in May 2009, left DOE and now consults for energy companies. “When she left, RE-ENERGYSE was gone, too,” says one DOE official. The Administration made it official by removing the initiative from its 2012 budget.

“There are broad STEM workforce-development programs across the federal government,” says Carl Wieman, associate director for science at the White House Office of Science and Technology Policy, in explaining the decision. “And there are plenty of other opportunities to improve STEM education.” Wieman, a physics Nobel laureate and science educator who joined the Administration last fall, says that RE-ENERGYSE “may have been comprehensive, but that also makes it complex to set up and make work. So the decision was that we’ll find something that Congress is happier with and move on.”

That something, Chu says, is a request for \$11 million in 2012 to more than double the number of graduate research fellows supported by DOE’s Office of Science. It’s the most direct way to beef up the nation’s clean-energy workforce, he says: “The pipeline starts early, by getting [elementary and secondary school students] interested in science. But [educating] undergraduates and those in graduate school, this is something that we feel very strongly about.” Some 150 fellows are now funded under a program begun in 2009. Last month, DOE also announced a competition this year to award 20 postdoctoral fellowships for research on clean-energy technologies.

—JEFFREY MERVIS

CREDIT: UPI PHOTO/LANDOV

NSF Hits Ceiling on Graduate Fellowships

The president’s 2012 budget request also suspends plans to triple the number of graduate research fellowships at the National Science Foundation (NSF).

Two years ago, President Barack Obama proposed a 5-year tripling of the foundation’s most prestigious awards for graduate students, to 3000 by 2013, as part of his promise to train more scientists and engineers. But instead of continuing to move toward that goal, NSF expects to hold the number of fellows steady next year at 2000 and to use a proposed 31% increase in the program’s budget to raise stipends and increase the subsidy to universities for enrolling them. The stipends for new fellows would rise starting in 2013 by \$2000, to \$32,000, while the so-called educational allowance given to universities hosting the fellows would grow starting next year by \$1500, to \$12,000.

NSF officials say that the stipend needs to be raised to keep the program competitive with other fellowships and that they wanted to ease the burden on universities, which they said are already heavily subsidizing tuition. Asked whether she foresees the number of fellows growing in the future, Joan Ferrini-Mundy, who heads NSF’s education directorate, says, “We can’t project beyond 2012.” Carl Wieman, associate director for science at the White House Office of Science and Technology Policy, says that “2000 is a more realistic level given the current budget situation.” —J.D.M.



PATENTS

Signature on Visitor's Form Fuels *Stanford v. Roche* Court Battle

Mark Holodniy didn't think he was making a big decision when he checked in as a visiting scientist at the biotech company Cetus near San Francisco, California, on Valentine's Day 1989. "That first day I was getting keys to the building, parking passes, signing forms, [that] kind of stuff," Holodniy recalls. Then 31, Holodniy had been sent by his lab chief at Stanford University, Thomas Merigan, to learn about Cetus's new molecular technique, the polymerase chain reaction (PCR). Merigan's group wanted to use it to monitor HIV infections. On arrival, Holodniy signed a pledge to honor company secrets and intellectual property rules: "My take on it was that I was signing a visitor's agreement and not that I was signing away my intellectual property rights for the rest of my life." But company lawyers say he actually did sign those rights away.

Twenty-two years later, Holodniy's signature on that form is at the center of a major patent fight before the U.S. Supreme Court. Lawyers representing Cetus and its successor, Roche Molecular Systems, say the young scientist forfeited not only his own patent rights but also those of his university. Stanford says this cannot be so. In its view, a U.S. law to encourage commercialization of research, the Bayh-Dole Act of 1980, directs that a university scientist on a U.S. research grant—as Holodniy was—must assign patent rights to the university. Furthermore, when Holodniy was hired by Stanford in 1988, he agreed to assign future patents arising from his work to the university.

The legal action traces its roots back to 1999, the year Stanford received the first of three patents on a technique that arose

out of the collaboration between Cetus and Merigan. They developed a method using Cetus's patented PCR process to quantify the amount of viral RNA in serum; the Stanford scientists focused on correlating the "viral load" with data from HIV patients. This led to a way to monitor disease progression and tailor drug regimens.

Both research groups contributed to the work. Cetus, in addition to developing PCR, devised an index of viral load. Stanford scientists Merigan, Holodniy, and David Katzenstein focused on the clinical data. But according to Stanford, Cetus "slept" on the intellectual property; the patents list only Stanford scientists as inventors.

In 1991, Cetus was taken over by Roche, a Swiss company. And in 1996, Roche began to manufacture and sell HIV monitoring kits, using the Cetus-Stanford approach. The kits now earn many millions of dollars a year. In 2000, Stanford started pressing Roche to take a license from the university and pay royalties. Roche ignored the university's demands, so Stanford sued Roche in 2005. Roche countersued, arguing that the patents were invalid for several reasons.

Two lower courts have considered the claims and given divergent opinions on who should prevail. A California district court ruled in 2007 that Stanford was right on one point: Bayh-Dole takes priority, barring university scientists on U.S. grants from making their own third-party patent deals, as Holodniy unwittingly did by signing the Cetus visitor's agreement. However, in a separate decision, the court also decided that Stanford's patents were invalid because they are obvious—a ruling that remains unresolved.

Viral load. A dispute over who owns rights to a key HIV assay has gone to the Supreme Court.

In 2009, the U.S. Court of Appeals for the Federal Circuit gave its opinion. It was silent about the patents' validity but agreed with Roche that Holodniy had given away his rights by signing the visitor's form. It said that Holodniy's earlier commitment in 1988 to give the patents to Stanford was not relevant because it was a mere promise. Stanford's form said, "I agree to assign," whereas Cetus's said, "I will assign and do hereby assign."

The court's rejection of the university's priority set off alarm bells. The big issue, Stanford attorney Patrick Dunkley says, is whether Holodniy's signature on a visitor's form may be used to "undermine" the entire Bayh-Dole system that encourages government-funded researchers to market their inventions. Unless the appeals court ruling is reversed, Dunkley says, it could render "30 years' worth" of patents under Bayh-Dole uncertain.

Major U.S. research universities have sided with Stanford, supporting its argument that academic-commercial collaborations could be put in question. The Association of American Universities—joined by more than 70 universities and seven research organizations, including AAAS (the publisher of *Science*)—filed a friend-of-the-court brief saying that Roche's arguments, if accepted, "would discourage continued collaboration between universities and private industry." The Obama Administration sided with Stanford, too. The U.S. Solicitor General wrote that the appeals court decision sets the Bayh-Dole system of commercialization "on its head" and "creates serious uncertainty" about who owns patents from government-sponsored research.

Several high-tech corporations and their representatives have sided with Roche, including Intel, the Pharmaceutical Research and Manufacturers of America, and the Biotechnology Industry Organization (BIO). The companies generally see it as a simple contract issue: Cetus's visitor's agreement must be honored. Roche argues that the company commercialized the discovery on its own and that Stanford did no more than attempt to profit by claiming an invention "retroactively." Roche and BIO both say that if the court sides with Stanford, entrepreneurs in the future will avoid working with government-funded scientists, particularly at universities.

The Supreme Court heard oral arguments on 28 February and is expected to deliver its decision by June.

—ELIOT MARSHALL

IMMIGRATION REFORM

Plans Afoot to Extend Welcome Mat To More U.S.-Trained Science Grads

It's relatively easy to get politicians across the spectrum to agree that the U.S. economy benefits from having foreign-born scientists and engineers remain in the country after earning an advanced degree from a U.S. university. They earn 37% of all U.S. Ph.D. degrees in science and engineering, for example, and create a disproportionate share of start-up companies. But accomplishing that goal without igniting the passions that traditionally have made immigration reform too hot for politicians to handle? That's a much harder job.

Last week, a key congressional panel heard proposals to retain the "best and brightest" foreign students without disadvantaging U.S. workers. Those who want to reform the system say they are heartened by the broad interest in the topic. The encouraging signs, they say, include President Barack Obama's State of the Union plea in January to "stop expelling talented, responsible young people who could be staffing our research labs or starting a new business" and bipartisan legislation introduced by Representative Jeff Flake (R-AZ), a Tea Party favorite, that would give Obama much of his wish. But they acknowledge that the approach of the 2012 elections means the political window of opportunity won't stay open for very long.

For foreign-born, U.S.-trained scientists who wish to remain in the country, the first order of business is to obtain a temporary work visa. Many graduates begin their professional careers by seeking an employer-sponsored temporary visa, called an H-1B.

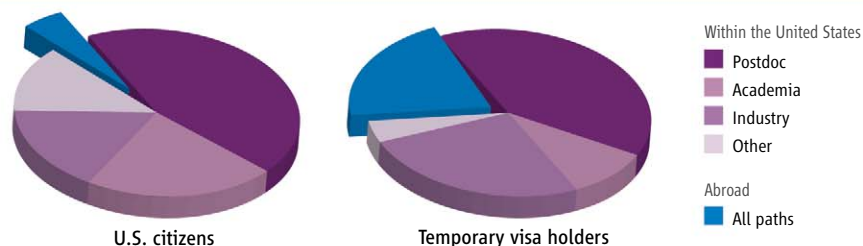
The H-1B visa, good for up to 6 years, was created in 1990 as a way station for those on the road to earning permanent residency status, or becoming "green card" holders. But critics of the program say far too few make the transition. They point to companies that use their power over these workers (63% of whom work in science and engineering fields) to keep wages low, restrict job mobility, and even force them to transfer their knowledge to colleagues based abroad. Another problem is that demand for H-1B visas historically has far exceeded supply; in boom times the annual cap, now 65,000, has been met within days of a new year.

The focus of the hearing, by the immigration panel of the House of Representatives Judiciary Committee, was on ways to

fix problems with the H-1B program. But it also provided a forum for those advocating a more direct approach to retain these scientists and budding entrepreneurs.

The most sweeping proposal came from a former congressman, Bruce Morrison, testifying on behalf of IEEE-USA, the lobbying arm of the 210,000-member Institute of Electrical and Electronics Engineers professional society, and the Semiconductor Industry Association (SIA), a trade group. Foreign-born workers with a master's or doctoral degree from a "quality" U.S. graduate program should receive priority status for obtaining a green card, he said, and their employers should be encouraged to apply as soon as they are hired.

SCIENCE AND ENGINEERING PH.D.S WITH DEFINITE PLANS AFTER GRADUATION



"The H-1B is not America's most effective welcome mat," Morrison says. Getting a green card would allow these workers to make full use of their talents, he argues. "With green cards you do not have to write endless rules regarding portability and prevailing wages."

That approach is similar in principle to that of Flake's bill (H.R. 399), the Stopping Trained in America Ph.D.s from Leaving the Economy Act. It would do what its acronym, STAPLE, implies: attach a green card to their diplomas. But Flake's bill, which would actually provide an exemption from the H-1B visa cap, is restricted to Ph.D. recipients, while the IEEE-SIA proposal would encompass a much larger talent pool. Representative Zoe Lofgren (D-CA), ranking member and former chair of the immigration panel, is drafting a bill that is expected to incorporate this idea, although she's still vetting suggestions from universities, industry, labor unions, and other stakeholders.

Creating a direct path from degree to citizenship would likely make U.S. graduate schools even more attractive to foreign

students. Some labor researchers worry that institutions with low standards might start graduate programs simply to attract paying customers, without regard to quality. "You lose your focus on education, for one thing," says B. Lindsay Lowell, a demographer at the Institute for the Study of International Migration at Georgetown University in Washington, D.C.

Lofgren agrees that "there has to be attention paid to the [quality of the] institutions themselves," and Morrison says, "I would not delegate to [immigration officials], who know nothing about science, the ranking of science graduate programs." Instead, he suggests turning to federal research agencies, whose competitive grants programs "have already identified the kinds of schools that have a track record of producing students with top-notch technical skills."

The fate of these and other immigration reform bills in the House lies with the chair of the full committee, Representative Lamar

Brain drain? Current immigration policies may be one reason new foreign-born Ph.D.s are more likely to take jobs outside the United States.

Smith (R-TX). Smith attended only part of the hearing and said little, but in his opening statement he flagged two possible tweaks to current policies. "It appears that doctorates lead to much more invention than bachelor's or master's degrees," he noted, adding that "if Congress doesn't act to increase the H-1B cap, then we may need to examine what sort of workers qualify."

Lofgren and many Democrats would prefer comprehensive reform. But Morrison, a Democrat whom ranking member Representative John Conyers (D-MI) chided for having gone "from a worker guy to a free marketer" in the 20 years since he left Congress, says small steps may be the price for consensus. "We'll have a better debate and more success on immigration if people can agree that immigration isn't the third rail," he says. "That's why we've waited 20 years to do things that we should have done in 20 minutes."

—JEFFREY MERVIS

SOURCE: NSF SRS, SURVEY OF EARNED DOCTORATES, 2008

SINGAPORE

Winds of Change Leave Bioscientists Scrambling

SINGAPORE—Over the past decade, this city-state has generated a lot of buzz by attracting international A-list scientists to its bioscience institutes. Much more quietly, Singapore's universities have been landing top researchers and rising in international rankings. Now, however, the fortunes of these two sets of institutions may be diverging.

In a 5-year plan launched this month, Singapore will boost public spending on research by 20% compared with spending during the previous 5 years. This largesse comes with a price: The government is demanding more economic bang from its research bucks. The drive to make science pay is falling hard on bioscience institutes under the Agency for Science, Technology and Research (A*STAR). Their core budgets will be cut and A*STAR's bioscientists, accustomed to assured funding, must compete for grants decided in part by the likelihood of an economic payoff. In contrast, government funding for research at the universities would increase steadily.

The sudden change has left many A*STAR scientists confused and chagrined. "Planning for the change has been rushed, the execution has been disappointing, and the messaging to the scientific community problematic," says Edison Liu, director of A*STAR's Genome Institute of Singapore. Some are packing up. Two high-profile scientists who arrived here with great fanfare 5 years ago—cancer researchers Nancy Jenkins and Neal Copeland—will return to the United States in September. Others say they are mulling exit strategies.

Singapore's new approach grew out of a yearlong review of the government's economic strategy, including its R&D investment. One outcome was a reaffirmation of the city-state's faith in research as a driver of economic growth. The government has vowed to raise public science spending to 1% of gross domestic product (GDP) by 2015. Projected spending will total \$12.6 billion over the next 5 years. Singapore is counting on private investment to raise total R&D spending from all sources to 3.5% of GDP in 2015, up from 2.3% in 2009. "It's a stretch," says A*STAR Chair Lim Chuan Poh. But achieving 3.5% would "put Singapore in the top 10 countries in research intensiveness."

To get the private sector to do its part,

A*STAR is tweaking the way it funds its 12 bioscience institutions. They will receive core support totaling approximately \$1.3 billion over 5 years, roughly 70% of previous levels. To maintain research efforts or expand in new directions, A*STAR's bioscientists must compete for grants from the new Industry Alignment Fund, which will dole out \$470 million over the 5 years. These grants "are linked to encouraging work toward industrial applications and bringing in R&D income to Singapore," says George Radda, a bioimaging specialist who chairs A*STAR's Biomedical Research Council. Bioscientists can also apply for other A*STAR grants that

to Southeast Asia.

To stimulate "world-class investigator-led research with a global impact," as NRF explains, the agency over the past 3 years has funded five Research Centers of Excellence in "areas aligned with the long-term strategic interests of Singapore." The centers will split about \$600 million over 5 years.

Two centers show how Singapore is broadening its research agenda. Geologist Kerry Sieh gave up a tenured position at the California Institute of Technology in Pasadena to be the founding director of Nanyang's Earth Observatory of Singapore. He says he was lured by Singapore's commitment to strengthening its universities, the challenge of building a program from scratch, and the opportunity to do pioneering fieldwork in Southeast Asia. And the Centre for Quantum Technologies at NUS is already recognized for its research on quantum information, says quantum theorist Stephanie Wehner, who joined the center's faculty last summer after finishing a postdoc at Stanford University in Palo Alto, California.

The support that Singapore's academics are enjoying is cold comfort to A*STAR's bioscientists. Many contacted by *Science* say they feel betrayed because the conditions that persuaded them to relocate to Singapore have suddenly changed. A common complaint is that the criteria for awarding Industry Alignment Fund grants are unclear. Another concern is how to sustain long-term projects launched on the assumption

of unstinting support. Several scientists suspect that Singapore is consigning basic investigator-led science to the universities while insisting that A*STAR, a department of the Economic Development Board, concentrate on mission-oriented research.

Radda agrees that the new strategy's rollout has hit snags, particularly with the Industry Alignment Fund's debut call for proposals in December. "We didn't give ourselves enough time, and I don't think we got that right," he says. He predicts that a second call for proposals in May will convince researchers that additional funding is not a mirage.

Still, Singapore's bioscientists must come to terms with the push for economic payoff. "It's no different from the rest of the world," Radda says. "All [funding agencies] are looking for what they are getting back for their research money."

—DENNIS NORMILE



"All [funding agencies] are looking for what they are getting back for their research money."

—GEORGE RADDA,
A*STAR'S BIOMEDICAL
RESEARCH COUNCIL

"Planning ... has been rushed, the execution has been disappointing, and the messaging to the scientific community problematic."

—EDISON LIU,
GENOME INSTITUTE OF SINGAPORE



support clinical research, for example, and for grants from other agencies, such as the National Research Foundation (NRF).

NRF and the Education Ministry, meanwhile, are supporting the National University of Singapore (NUS) and Nanyang Technological University in their quest to become global research university powerhouses. Over the past decade, both schools beefed up their faculties and research, and expanded graduate programs. "Science here has undergone a revolution in the past decade," says Paul Matsudaira, a bioimaging specialist who left the Whitehead Institute in Cambridge, Massachusetts, in 2009 to head NUS's biology department. In another sign of the evolution of Singapore's universities, NUS and Yale University announced last week that they will establish Yale-NUS College in Singapore to bring a new approach to liberal arts education

Can Biotech and Organic Farmers Get Along?

The U.S. Department of Agriculture has been taking a closer look at the impact of biotech crops on organic farms. Research is providing tools to help them thrive side by side, but the politics are tricky

FOR PROPONENTS OF AGRICULTURAL BIOTECHNOLOGY, getting new crops to farmers has become a lot more complicated in the past few years. Take alfalfa, genetically modified (GM) to resist herbicides. This long-awaited variety was introduced in 2005, and within a year, farmers snapped up all the available seed and sowed 250,000 hectares. But in 2007, all planting was halted after opponents persuaded a federal judge to order the U.S. Department of Agriculture (USDA) to conduct a more comprehensive environmental review—one that was completed only this past December.

And then there are sugar beets. USDA approved herbicide-resistant sugar beets in 2005. About 3 years later, environmental groups and seed producers sued USDA, again claiming an inadequate environmental impact assessment. In August 2010, the court prohibited planting until the agency completes a more detailed review next year. In February, the court lifted the ban, but the confusion and uncertainty have thrown the industry into turmoil. Both of these cases have meant extra regulatory work at USDA, contributing to a growing backlog of applications for biotech approval. Other lawsuits have hit the industry directly in the wallet: Last year, a court ordered Bayer CropScience to pay \$48 million in damages after experimental transgenic rice accidentally entered the food supply.

This terrain is a stark contrast to the boom years of the 1990s, when varieties of GM crops quickly spread across the United States. Although opponents raised concerns about safety and ecological impacts, USDA approved variety after variety. Philosophical

objections to biotech crops haven't diminished, but the current spate of lawsuits and delays now hinges on economic damages; the issue is how to keep the transgenes in GM crops from spreading to organic crops, which can be costly for organic farmers to prevent. Failure can mean they give up the premium price that organic products fetch.

Enter Tom Vilsack, head of USDA, whose new mantra is "coexistence." In his vision, biotech continues to innovate, organic farmers are protected from unwanted transgenes, and no one is tied up in costly litigation. In December, Vilsack electrified the debate by proposing the agency's first restrictions on biotech growers—specifying isolation distances between GM and organic alfalfa to prevent gene flow—and hosting a high-level discussion of how to achieve coexistence. For the organic community, the moves were long overdue. Although to the community's dismay, USDA subsequently reapproved GM alfalfa without any restrictions, the agency pledged to fund research on coexistence and to reinstate a key advisory committee on biotechnology to provide advice on the issue.

Experts say without question that GM and organic farmers can coexist—if there is an achievable, agreed-upon standard of how pure is pure enough to receive the organic label or to be accepted by overseas markets. At this stage, with so much of U.S. fields planted with GM crops—93% for soybeans—everyone

agrees it's impossible to completely exclude transgenes from organic fields, but they can be kept to minimal levels. "As long as zero tolerance is insisted upon, you can forget about coexistence," says Patrick Byrne, a plant geneticist at Colorado State University in Fort Collins.

With a defined threshold, say the presence of 0.1% or 0.9% transgenic seeds, depending on the crop, scientists can figure out the appropriate distances between fields to minimize gene flow. In the future, computer models of pollinator behavior may help provide recommendations tailored to particular landscapes. Another approach to prevent the spread of transgenes is to breed crops that can't be fertilized by transgenic pollen; the first commercial varieties of corn with this protection should be released this fall.

Harmony isn't likely anytime soon, however. The sides remain split on key issues. Organic groups demand more government oversight, that the biotech industry share the cost of preventing gene flow, and the creation of a compensation fund for damages if their crops cannot be sold as organic. The biotech industry opposes all of these goals. So far, USDA seems to continue to lean toward the industry in how it approves GM crops. "The public face of USDA is changing, but their actions are business as usual," says A. Bryan Endres, an expert in agriculture law at the University of Illinois, Urbana-Champaign.

A changed landscape

Coexistence has been a part of agriculture for a long time. Neighboring farmers have had

Online
sciencemag.org



Podcast interview
with author
Erik Stokstad.

Battleground. Alfalfa seed farms in the Willamette Valley, Oregon, were at the center of a protracted legal fight over genetically modified crops.

to make sure that they keep enough distance when they plant crops that shouldn't cross-pollinate, such as popcorn and sweet corn, lest they end up with something unsalable. The issue is even more important when growing seeds for planting, because seed purity affects the quality and consistency of the crops. For seed growers, USDA and certifying organizations have for decades provided guidance on best practices, including isolation distances for fields.

But the advent of biotech changed the game because DNA tests make it easy to find GM seeds—at very low concentrations. “The transgenes have given a great opportunity for finding that contamination,” says Carol Mallory-Smith of Oregon State University (OSU) in Corvallis, whose research has shown just how easily transgenes move around (see p. 168). And given the strong opposition to GM crops in some export markets, Japan and Europe, especially, farmers are worried about not being able to sell specially grown organic crops if just the tiniest trace of transgenes is found. “The organic industry is extremely concerned” about the spread of transgenes, says Christine Bushway, executive director of the Organic Trade Association in Washington, D.C.

There are few data on the economic impact of approved GM crops on organic farmers, but they say the burden can be steep. Organic farmers bear the costs of preventing gene flow into their crops, which they do by planting buffer strips, for example, and testing for transgenes. Economists point out that these costs are generally compensated by the premium price fetched by organic crops. However, organic farmers can face other risks as well; planting at different times than their neighbors, a tactic to avoid gene flow, can lower yields and raise the odds of crop loss from bad weather.

Coexistence strategies are based on how pollen moves, which varies dramatically by crop. Soybeans, for example, are self-pollinating, and insects only rarely spread their pollen. Corn, which is outcrossing and wind-pollinated, is another matter entirely.

To figure out what isolation distances and farming practices to recommend to farmers dealing with GM crops, scientists typically sow a field with plants that have a genetic marker, such as herbicide tolerance. Then they plant nontransgenic seeds in “study plots” at various distances. The transgenic crops will survive when sprayed with herbicide; if any plants in the study plot are still living, then they have acquired the resistance gene from the original field.

Although such experiments are straightforward in principle, they rarely encompass the diversity and complexity of agricultural



On the trail. By dusting honeybees with colored powder, researchers can track how far they spread pollen.

and environmental conditions. Farming practices, geography, and weather can all influence the spread of pollen. This is especially true for the dynamics of insect-pollinated crops, such as alfalfa. Seed producers may bring in hives full of honeybees or leafcutter bees, which fly different distances, to pollinate their crops. Native pollinators such as bumblebees may also influence how far the pollen travels. Researchers with the University of California, Davis, led by Larry Teuber, and USDA recently spent 3 years tracking pollinators (see photos, above) and sampling seed to measure gene spread via pollen. The results are being used to verify and refine isolation distances

for biotech alfalfa.

In a different project, Johanne Brunet, an evolutionary biologist with the USDA's Agricultural Research Service in Madison, Wisconsin, is building a model to predict gene flow based on pollinator behavior and the agricultural landscape. “My hope is that it might help us design better ways to prevent gene flow,” Brunet says. Ultimately, the model might provide isolation distances and other guidance customized for particular farms and crops.

Pollen barriers

Isolation distances can't prevent gene flow entirely, however. Wind and insects can take pollen surprisingly far, albeit in small amounts (*Science*, 28 June 2002, p. 2314). So rather than simply focusing on changing farming practices, some scientists are investigating another strategy: changing the plant itself. The hope is that by designing plants that can't be fertilized by transgenic pollen, researchers can “fence out” GM crops.

For instance, researchers have known for decades that some varieties of tropical corn can't be pollinated by other varieties, a phenomenon known as cross incompatibility. Fertilization of these varieties can take place only if the pollen and the plant receiving it have genes called *GA1* and *GA2*. About 10 years ago Tom Hoegemeyer, now at the University of Nebraska, Lincoln, began breeding this trait into regular field corn, which he dubbed PuraMaize. After 4 years of further breeding, Blue River Hybrids Organic Seed, a company in Kelley, Iowa, plans to start selling three commercial hybrid varieties of PuraMaize to organic farmers this fall. “We believe it should be effective,” says company founder Maury Johnson, who has done small-scale tests.

But it's unlikely to be a long-term solution because even though PuraMaize plants are protected from foreign genes, their own pollen is able to fertilize other varieties. This means that the *GA1* and *GA2* genes can spread, mixing into the DNA of other corn. If that happens with transgenic corn, it would gain the ability to pollinate PuraMaize. It's also possible that *GA1* and *GA2* could slip into future kinds of GM corn from other research varieties used during breeding. “This [PuraMaize] system will probably break down,” predicts Major Goodman, a corn breeder at North Carolina State University in Raleigh, who is nevertheless working on adding the trait into other varieties of corn.

Another source of cross incompatibility is *teosinte crossing barrier-1* (TCB). This locus, which behaves similarly to *GA1* and

Continued on page 169

Honest broker. Mallory-Smith is a key player in the legal tussles over genetically modified crops.

PROFILE: CAROL MALLORY-SMITH

Scientist in the Middle of the GM-Organic Wars

For Carol Mallory-Smith of Oregon State University in Corvallis, the migration of genes in agricultural crops is not just a research topic or a matter of policy debate. It's the cause of a vexing quarrel among her neighbors: the farmers of Oregon's Willamette Valley.

This valley, because of its mild climate and ample water for irrigation, is one of the world's great seed-growing centers, supplying farms, gardens, and golf courses worldwide. Because pollination is at the heart of seed production, the valley is now the scene of heated debates—and one far-reaching lawsuit—over the consequences of genetically modified pollen or seeds drifting into fields filled with sexually compatible non-GM crops. Organic farmers who fear contamination of their crops are on one side; the growers of seed for genetically engineered sugar beets are on the other. And Mallory-Smith is in the middle. Her publications have found an avid readership among biotech industry lawyers and activists opposed to GM crops. "The research part has been fantastic," she says with a wry smile. "The politics of it is difficult, to say the least."

Mallory-Smith came to the topic of migrating modified genes through her research on cross-pollination between wheat and one of its close relatives, a weed called jointed goatgrass. The two species can produce hybrids, and Mallory-Smith wanted to know whether this would allow new herbicide-resistance genes that had been introduced into wheat to migrate into the crop's weedy relative.

She extended that research to turfgrass in 2001, when the Scotts Miracle-Gro Co. began experimental field trials of glyphosate-resistant, sometimes called "Roundup Ready," creeping bentgrass near the town of Madras, in central Oregon. Mallory-Smith began monitoring areas near the fields and, along with other researchers, documented a large-scale genetic migra-

tion: Hundreds of glyphosate-resistant grass plants evaded all the company's efforts to confine them. This grass still has not been approved for unrestricted cultivation, so the company is supposed to find and destroy any such plants that show up outside of its trial plots. Some GM grass was found 20 kilometers from the fields where it belonged. It was destroyed, but years later, researchers continue to find unapproved GM grass plants growing wild near the former test plots in Madras.

Much of this gene jailbreak probably happened on one day in 2003, when an unexpected windstorm blew away swaths of seed that had been left out to dry at the Scotts research facility. Mallory-Smith says this event should not have been a surprise, because freak weather is a natural part of farming: "This is what happens in production agriculture."

That episode cemented Mallory-Smith's reputation as an expert on migrating transgenes, especially in the Willamette Valley. When sugar beet seedlings turned up in topsoil that was sold at a garden supply store in Corvallis, residents brought them to Mallory-Smith for testing. The seedlings turned out to be glyphosate-resistant.

Last fall, farmers in eastern Oregon noticed that some grass in their irrigation ditches seemed immune to Roundup. They, too, sent samples of the grass to Mallory-Smith, who confirmed that the grass contained an inserted glyphosate-resistance gene. She also flew out to take a look for herself. "The plants have obviously been there for a while. They're large. They've gone to seed. My guess is, they're 3 or 4 years old," she says.

Such grass still cannot be legally planted without a permit from the U.S. Department of Agriculture (USDA). Most likely, it spread from another set of field trials conducted by Scotts Miracle-Gro just across the border in Idaho.

The Scotts Miracle-Gro Co. tried to elimi-

nate the rogue grass over the past few weeks with a herbicide that state regulators approved temporarily for this purpose. (Glyphosate had been the only weed killer allowed in irrigation ditches.) But according to Jay Chamberlin, manager of the Owyhee Irrigation District in Nyssa, Oregon, no more spraying will be allowed this spring because water is about to reenter the ditches. Chamberlin says it is likely that some unapproved turfgrass will remain in the ditches through the summer, and it may spread farther. "The more they look, the more they find," he says.

These experiences convinced Mallory-Smith that USDA regulators haven't fully understood the dynamics of gene flow, at least when it comes to turfgrass. "They knew a lot about corn, soybeans, and cotton. But now we're dealing with a perennial, with lots of relatives. And it's weedier; it does survive outside of cultivation. They didn't have an understanding of that kind of cultivation," she says.

Generally, she says, regulators and biotech companies have been overly confident that they can prevent modified genes from spreading. "When you put them out there, you have to accept the fact that you're not going to contain them; you're not going to retract all these genes," she says.

For all the shortcomings that Mallory-Smith sees in GM regulation, she doesn't really fit in the anti-GM camp. "She's an honest broker, if there are any honest brokers in all this," says Steven Strauss, a colleague at Oregon State. Mallory-Smith says she's sympathetic to both sides. "I'm a public servant, and that sometimes means that you end up in the middle of these factions," she says. But she's hoping that both organic farmers and growers of genetically engineered beets can thrive: "We need all our industries."

—DAN CHARLES

Dan Charles is a writer based in Washington, D.C.

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GA2, was first researched in the 1970s by Jerry Kermicle of the University of Wisconsin (UW), Madison. Kermicle has taken a first step at breeding TCB into regular corn, but so far there hasn't been much interest from corn breeders. "There's nothing in it for the biotech firms," he says, "and the small players in the organic community, they're not organized or centralized."

Efforts are also under way to modify biotech crops so that they can't spread pollen. This would essentially "fence in" the transgenes. Several approaches are being investigated in labs, such as using RNAi to make transgenic plants sterile. But such approaches are fairly far away from development and implementation, largely for commercial reasons, says Henry Daniell, a molecular biologist at the University of Central Florida College of Medicine in Orlando: "The biotech companies have no incentive unless they get a strong product advantage."

At least one company is pursuing a technology that would, as a side benefit, prevent gene flow in pollen. That approach, which Bayer CropScience has researched in soybeans and other plants, is to engineer chloroplasts to express transgenic traits. Because chloroplasts are maternally inherited, pollen very rarely carries the transgene. And traits placed into chloroplasts can be expressed at levels higher than those put into the nuclear genome, Daniell says, although it is a more difficult approach. Steven Strauss of OSU notes that this approach would be most useful as part of a suite of containment tools for biopharmaceuticals rather than food crops.

Some in the organic community are skeptical in any case. "We do not believe there exists a technological silver bullet to the multiple challenges that genetically engineered crops present," says Kristina Hubbard, director of advocacy for the Organic Seed Alliance in Port Townsend, Washington.

Entrenched positions

Even if gene flow from GM to organic crops could be prevented in the field, there are ample opportunities for it to occur after harvest, for instance, through accidental mixing. This occurred with dramatic consequences in September 2000 with biotech corn called StarLink. Approved only for animal feed, StarLink was detected in taco shells and then found in more than 10% of the corn supply. Massive recalls resulted. The debacle was estimated to

have cost Aventis, the developer of StarLink, hundreds of millions of dollars.

Commingle of approved biotech varieties won't lead to recalls of organic food in the United States. That's because, even though most consumers are unaware, organic products in the United States are allowed to contain transgenes. To earn the organic label, USDA specifies that farmers can't plant GM crops. But if transgenes accidentally end up in their fields, the harvest can still be called organic.

That's unacceptable to some buyers, who have zero tolerance for GM organisms (GMOs). But others recognize that a little contamination is inevitable, which underscores the importance of agreeing on quantitative standards for how pure is pure enough.

Such standards can be set in a variety of ways. Some countries decide what percentage of transgenes is acceptable in imported commodities, say 1% in soybeans. But in the

less than 0.1% transgenes in their fields. Forage Genetics International (FGI) in Nampa, Idaho, which commercialized biotech alfalfa with Monsanto, will require farmers who buy its seed to agree to its list of best practices. "The stewardship programs are mandatory," says FGI President Mark McCaslin.

But such agreements aren't enough for some organic groups, which would like to see the government, relying on a scientific advisory committee, set such standards and create penalties for breaking them. USDA doesn't seem headed in that direction. In its January decision on alfalfa (*Science*, 4 February, p. 523), the agency opted to leave regulation up to industry, as it did the next month when it approved the first corn variety designed for ethanol producers. "At the place we are now politically, I don't think we'll see strict standards put in place" by USDA, says Alison Peck of the West Virginia University College of Law in Morgantown.

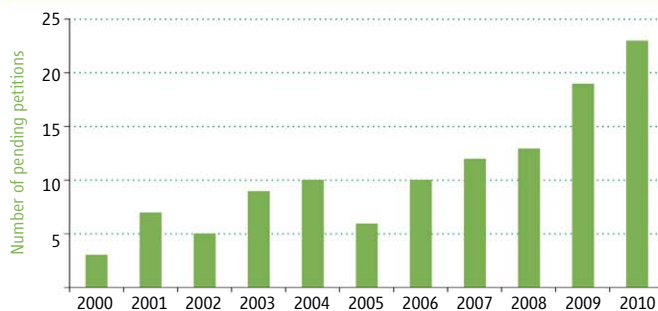
The organic community may yet get at least a discussion of its most controversial wish: a liability fund that would compensate growers who can't sell to markets that reject biotech. This idea is anathema to the biotech industry, which argues that shifting this cost to technology developers would stifle innovation. Vilsack, however, has asked the newly reestablished USDA Advisory Committee on Biotechnology and 21st Century Agriculture to explore the concept.

As for Vilsack's goal of reducing litigation, most observers say there's probably no way to stop the lawsuits against USDA, which are filed by advocacy groups—such as the Center for Food Safety, the lead plaintiff in the alfalfa and sugar beet lawsuits—that remain vehemently opposed to new GM crops and want existing varieties yanked as well. "Expect to see more litigation," the University of Illinois's Endres says.

Even so, many are hopeful that the new discussions, taking place among farmers, seed producers, and buyers, will lead to concessions that lower the risk for organic farmers. "I'm optimistic that a lot of people in places of influence are thinking about this," says Manjit Misra of Iowa State University in Ames. Molly Jahn of UW Madison is also sanguine, based on recent discussions with stakeholders involved in the alfalfa guidelines. "It's possible for reasonable people to commit to a way forward, grounded in science, with reasonable standards," she says.

—ERIK STOKSTAD

USDA'S BIOTECH BACKLOG



Under strain. More biotech crops are now stuck in regulatory review, partly from the time USDA is spending assessing impacts on organic farmers.

United States, the government has not set a standard for organic labels.

In the absence of a federal standard, a private labeling scheme, the Non-GMO Project, set its own targets of 0.25% for seeds and 0.9% for food and ingredients in 2008 for all participating products. Manufacturers who are certified as testing their ingredients for transgenes can use the Non-GMO Project label. "Doing this proactively with a high level of transparency was [decided to be] the best way to win and sustain consumer confidence" in organic products, says Charles Benbrook of the Organic Center in Boulder, Colorado, who is a technical adviser to the Non-GMO Project.

In addition, some agricultural trade groups in the United States have agreed on realistic standards for specific crops. Before GM alfalfa was first approved, for example, industry funded research into gene flow and determined that organic seed producers would be able to easily achieve a goal of

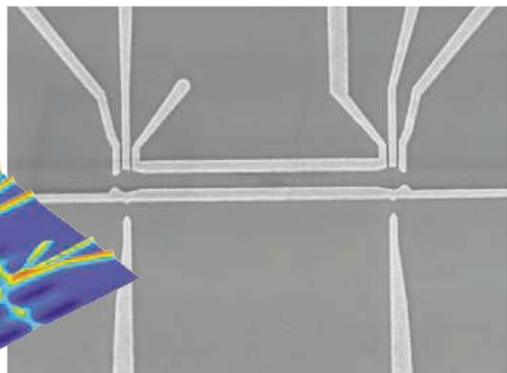
Electrons Surf Sound Waves To Connect the Quantum Dots

Physicists at the University of Cambridge in the United Kingdom have used sound waves to transport individual electrons over significant distances between artificial atoms on a chip. The technique might someday help scientists to shuttle information around within a quantum computer, although such a device is still far from reality. More immediately, the technique represents a striking confluence of electronics, acoustics, and nanotechnology.

The mock atoms are quantum dots, which can be either nanometer-sized bumps of semiconductor or simply spots on the surface of a chip ringed by electrodes that control them. As in an atom, an electron in a dot can occupy only a few quantum states of definite energy. Scientists can use a dot as a quantum bit, or “qubit,” to store information. For example, they can trap an electron in one state and use its spin to encode information: a “0” if the electron’s spin points up, a “1” if it points down, or—thanks to the weirdness of quantum mechanics—both 0 and 1 as the electron spins in both directions at once.

Researchers have used such qubits to perform elementary logic calculations. But they still face the problem of making the dots communicate with one another. One scheme involves getting an electron from one dot to “tunnel” into a neighboring dot. But that requires placing the two dots within a couple of hundred nanometers of each other, says Hendrik Bluhm, a physicist at RWTH Aachen University in Germany. “Moving quantum information around over long distances is a big problem for the field,” he says.

Now Robert McNeil, Christopher Ford, and colleagues at Cambridge have found a way to transport individual electrons much farther using sound waves. On a wafer of gallium arsenide, they laid out electrodes 4 micrometers apart that defined two quantum dots and also a channel between them. They also laid out a ladderlike electrode that, when charged, causes the gallium arsenide to contract, setting off a sound wave that ripples across the surface. The sound wave in turn



Conveyor. A sound wave (left) zips between the dots formed by the electrodes above.

produces a corrugated electric field that travels with it and can predictably pick up an electron from one dot and transfer it to the other. Literally surfing along at the speed of sound, the electron covers the distance between the dots in 1.5 nanoseconds, McNeil reported. “This procedure can be repeated hundreds of times with the same electron,” he says.

The next challenge is to ensure that the sound wave transporting the electron does not obliterate the delicate quantum state of the electron’s spin. The electron acts a bit like a gyroscope, and if the process is gentle enough, then transporting it across the semiconductor should simply make the direction of its spin turn, or “precess,” by a predictable amount, Bluhm says. McNeil says he and his colleagues are now trying to verify that it does.

But even without answering that question, the Cambridge team has performed a very pretty experiment, Bluhm says: “Just the bare technique is pretty impressive.”

Ice Is Predicted To Be Weirder Still

Plain old water continues to surprise physicists. For example, researchers have found that the molecules in pressurized ice can arrange themselves in at least 15 distinct crystalline patterns. Now a team of theorists says a film of ice only two molecules thick may form the oddest of all crystalline structures, a so-called quasicrystal, which lacks

the exact repeatability of an ordinary crystal structure but preserves other symmetries of a crystal.

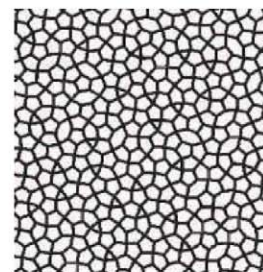
A quasicrystal is nature’s way of finessing a problem in geometry. Rows of squares, regular triangles, or regular hexagons can tile a plane. But equal-sided pentagons need not apply, as the five-sided figures leave gaps between them.

In the 1960s, mathematicians found a surprising way to skirt this rule with not-quite-regular patterns containing pentagons. The patterns don’t repeat from row to row. However, as with squares, triangles, and hexagons, the patterns look the same when rotated by a certain amount—in this case, a fifth of a turn. In the 1980s, scientists found alloys such as aluminum lithium copper that form such quasicrystal structures. Now, Valeria Molinero, a theoretical chemist at the University of Utah in Salt Lake City, and colleagues predict that a layer of ice under the right conditions should also form the bizarre structure.

Ice on surfaces has pulled some tricks before. For example, in bulk ice, water molecules arrange themselves in a three-dimensional pattern. But theorists had predicted that when confined to two layers on a surface, the molecules would bend so that they could lay down completely flat in a hexagonal pattern. And that’s what experimenters observed in 2009.

This time, Molinero and colleagues considered two layers of water molecules that are confined between two generic metallic surfaces and subjected to low temperatures and high pressures. Near the surface, the V-shaped molecules, with an oxygen atom between two hydrogen atoms, must balance several influences. The oxygen atoms prefer to sit atop the atoms in the surface. The molecules also try to maintain the lengths of their oxygen-hydrogen bonds and to arrange themselves so that none of those bonds is left dangling. Simulations that account for those factors reproduce the known phases of ice on surfaces and predict the existence of a quasicrystal, Molinero said at the meeting.

The prediction seems plausible to Angelos Michaelides, a chemist at University College



Thin ice. Two layers of water molecules are predicted to form this quasicrystal.

CREDITS (TOP TO BOTTOM): ROBERT MCNEIL/UNIVERSITY OF CAMBRIDGE (2); J. C. JOHNSTON ET AL., JOURNAL OF CHEMICAL PHYSICS 133 (21 OCTOBER 2010)

London. In fact, in 2009 he and his colleagues observed isolated chains of water molecules arranged in pentagons on a copper surface. “Increasingly, I’m starting to think that in two dimensions, the pentagons are more stable than the hexagons,” he says.

Greg Kimmel, a physicist at Pacific Northwest National Laboratory in Richland, Washington, says the key to making the quasicrystal will be finding a surface that holds the water tight enough that there’s no need for a second surface pushing down from above. That’s how his team produced the hexagonal pattern, which was also predicted assuming a squeeze between two surfaces.

One Cool Way to Erase Information

When your computer erases a bit of information—a “0” or a “1” stored in some electronic switch—it must generate a minimum amount of heat. Or so says a principle of thermodynamics. However, a tiny system can sometimes dip below that limit while erasing information, physicists from Canada report.

The finding won’t lead to infinitely cool computers, as on average the tiny bit still obeys the rule. But the exceptional events underscore the importance of fluctuations in very small systems and could point to a mechanism that cells might naturally exploit.

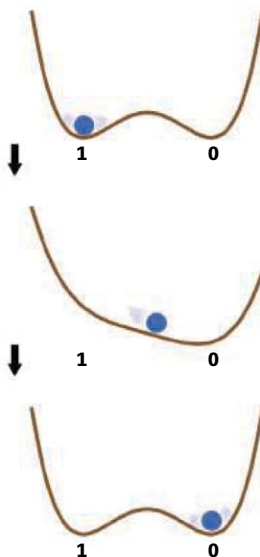
“That’s fantastic!” says Eric Lutz, a theorist at the University of Augsburg in Germany, who with a colleague predicted 2 years ago

how the thermodynamic limit might be evaded. “I tried to convince a number of people to do it.”

Disorder and information are the yin and yang of thermodynamics. Whenever a device destroys information, the disorder in the device and its surroundings must increase. That increase in disorder, or entropy, equals the release of heat. According to the so-called Landauer limit, erasing a bit at room temperature generates at least 0.003 billion-billionths of a joule of heat.

Strictly speaking, however, the Landauer limit applies to devices so large that the random jiggling of their atoms and molecules can be neglected when analyzing how they function. Things aren’t so simple in very small widgets. To prove it, Yonggun Jun and John Bechhoefer of Simon Fraser University in Burnaby, Canada, fashioned a bit out of a 200-nanometer-wide polystyrene bead suspended in water and surrounded by four electrodes.

Using a microscope, they tracked the position and speed of the bead, which was so small that it was buffeted by the jiggling water molecules. The electrodes effectively produced a landscape of voltage resembling a valley with two low spots (see diagram). If the bead started out in the left dip, then the bit was set to 1. If the bead started in the right dip, then the bit was set to 0.



Forget it. A tiny bead sits in one of two spots in a voltage landscape to record a 0 or 1. Nudging it to 0 erases information and can generate less heat than theoretical limits.

Starting with random settings, the researchers repeatedly erased the bit by lowering the voltage hump between the dips, tilting the whole valley to the right and restoring the original configuration, which forced the bead into the 0 spot. By tracking the bead, they could calculate the heat it generated, Jun reported.

As Lutz predicted, during some cycles it generated less heat than the Landauer limit demands because the water molecules helped nudge it along.

What’s it good for? One problem in applying the result is that it’s impossible to predict when the bit will beat the Landauer limit. However, Bechhoefer speculates that cells might exploit such fluctuations to drive the “molecular motors” within them more efficiently.

Lutz, who was not at the meeting, notes that today’s computer bits generate at least 100 times more heat than the Landauer limit. But if computer bits continue to shrink, then 20 or 30 years from now they may approach the limit, he says. Then hardware engineers will have to worry about thermal fluctuations spontaneously erasing information.

—ADRIAN CHO

Snapshots From the Meeting >>

Bang the drum slowly. For the second time, physicists have lowered the energy of a vibrating widget enough to achieve the least motion allowed by quantum mechanics: the so-called ground state of motion. Researchers had previously cooled a tiny diving



board-shaped gizmo to a few thousandths of a degree with a liquid helium refrigerator. This time, John Teufel of the National Institute of Standards and Technology in Boulder, Colorado, and colleagues borrowed a technique from laser physics and used microwaves to draw energy out of a tiny drumhead. The new widget oscillates 1000 times more slowly than the first quantum machine and should remain in a quantum state of motion much longer.

The power of true believers. If 10% of the members of a group

hold an unshakable conviction, their view will eventually win out, according to a network model. Theorist Sameet Sreenivasan and colleagues at Rensselaer Polytechnic Institute in Troy, New York, find that the structure of the group’s social connections does not greatly affect that threshold. Beyond the threshold, as the size of a group grows extremely large, only then do belief and disbelief coexist—assuming there are no unpersuadable disbelievers. Beate Schmittmann, a theorist at Virginia



Polytechnic Institute and State University in Blacksburg, says the analysis underscores that “if you want to have a diverse society, it has to be a certain size.” The U.S. civil rights movement took off as the percentage of African Americans approached 10% of the population, Sreenivasan notes. —A.C.

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The Robert B. Daugherty Water for Food Institute is bringing scientists, farmers, policymakers and industry leaders from around the world to Lincoln, Nebraska, May 1-4. They'll discuss research, guidelines and emerging technologies for managing water resources in an efficient and effective manner. The need to grow more food using less water has never been more vital.



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“A dream told me to do it.”

*Dr. Carl Alving on his inspiration
for inventing the vaccine patch.*



Carl R. Alving, M.D.
Chief of the Department of
Adjuvant & Antigen Research,
Division of Retrovirology at
the Walter Reed Army
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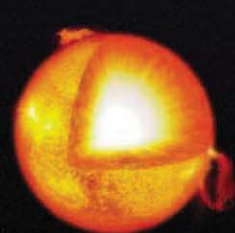
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LETTERS

edited by Jennifer Sills

Food for Thought on Climate Policy

IN THEIR POLICY FORUM "LINKING POLICY ON CLIMATE AND FOOD" (25 February, p. 1013), H. C. J. Godfray *et al.* argue that climate change negotiations must integrate policy to address both climate change and food insecurity. I would like to add two points.

First, avoidable transcontinental food trade should be minimized. For example, the U.S. aquaculture industry currently meets only 5 to 7% of domestic demand for seafood; the transportation required to import additional seafood has a substantial indirect CO₂ footprint (1). Agricultural policies should encourage sustainable local food production in an effort to reduce CO₂ emissions. A study that compared CO₂ emissions from the conventional long-distance import system with the emissions from Iowa-based regional and local systems found that the conventional system released between 5 and 17 times more CO₂ from the burning of fuel than did the regional and local systems (2). Similarly, rather than shipping food to underdeveloped nations, developed nations should leverage agricultural technologies and agricultural infrastructure investment to improve local food production.

Second, we must make use of food and feed ingredients in the byproducts of the growing biofuel sector (1). It is estimated that 100 million tons of proteins will be produced as coproducts of biofuel

Food transport. Is there a more climate-friendly approach?

biomass (3). Given that protein energy malnutrition is a leading cause of deaths of children in famine-stricken underdeveloped countries (4), the high-quality protein from biofuel industries (such as algae, cellulose, and lignocellulose) should be used as food (5). **BOBBAN G. SUBHADRA**

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AIDS Prevention Plans Must Reflect Local Values

IN HIS NEWS STORY "NO OPIATE SUBSTITUTES for the masses of IDVs" (special section on HIV/AIDS in Europe, 9 July 2010, p. 165), J. Cohen summarized an interview with me as follows: "Alexey Mazus, head of the city-sponsored Moscow Centre for HIV/AIDS Prevention and Treatment, articulates the government position—which he shares—and makes it clear that he strongly objects to other countries criticizing his country's stance toward harm reduction. 'It's not their business what's going on in the Russian Federation,' says Mazus." I would like to emphasize that I was expressing my personal opinion and that I am not in an official capacity to articulate the government position. Moreover, this quote misrepresents my views. Contrary to what was ascribed to me, I think that the global AIDS epidemic in general and epi-

demics in any particular country, including Russia, are everybody's business and that the response to those epidemics should be discussed, praised, and criticized.

However, it is true that I strongly disagree with the idea that prevention programs must be consistent across countries. Local circumstances, ethics, and cultural values must be taken into account, as was stated in the Declaration of the 2006 UN General Assembly special session on HIV/AIDS. In my opinion, harm reduction measures cannot be effective in Russia, and I cannot agree with the pressure on Russia to adopt this program based on the fact that it was effective in some other countries. In this case, my opinion coincides with the Russian state policy, but I speak only as an expert on HIV and AIDS epidemics in Russia.

ALEXEY MAZUS

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NSF Program Benefits Schools in Need

WE ARE AMONG THE MANY SCIENTISTS "shocked and upset" by the recent decision by the U.S. National Science Foundation (NSF) to terminate the successful GK-12 (Graduate Science, Technology, Engineering, and Mathematics Fellows in K-12 Education) program ("Outrage greets NSF decision to end STEM Fellows program," J. Mervis, *News & Analysis*, 4 March, p. 1127). Our use of these funds provides one example of the program's value.

Our program deploys graduate student Fellows to rural Oregon elementary schools, where they live as scientists-in-residence with hosts for 2 weeks per academic quarter, during which they co-teach children alongside teachers, implementing professionally developed inquiry-based science curriculum kits (lesson plans, materials, and equipment).



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Because these schools are far from universities and other centers of science or technology, the GK-12 Fellows are in many cases the only scientists the students have ever met. The teachers also have poor connections to experts and have little prior training in teaching hands-on, inquiry-based science; working with Fellows is crucial for adopting tools that will be effective after the Fellows' departure. The program benefits the Fellows by providing valuable training in communication and organization.

Ours is just one of many GK-12 programs nationwide that share the goal of improving science education. We find it remarkable that the entirety of GK-12 will be terminated, with focus redirected to "NSF programs that... partner academic scientists with local school districts." The local environs of universities are arguably the places least in need of additional outreach efforts.

The publicly funded U.S. research infrastructure has been extremely successful in advancing scientific knowledge, but largely unsuccessful in developing a public understanding of science. The nation needs more, not fewer, programs like GK-12.

RAGHUVeer PARTHASARATHY,*

DEAN LIVELYBROOKS, DAVID JOHNSON,
CATHERINE PAGE, SHANNON BOETTCHER,

J. DAVID COHEN, ERIC CORWIN, MIRIAM DEUTSCH,
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Drug Regulatory Systems Must Foster Innovation

WE SUPPORT M. A. HAMBURG'S PLEA FOR increased funding of regulatory science ("Advancing regulatory science," Editorial, 25 February, p. 987). We also share her ambition to modernize the regulatory system and bring 21st-century science and technology to drug development and drug evaluations.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

In addition, regulatory science should evaluate and study regulatory systems in terms of their ability to ensure patient safety, enhance public health, and stimulate innovation (1–3). During the past decades, the introduction of new innovative drugs has dropped, despite impressive investments and progress in biomedical research and development. Although the reasons for this innovation deficit are not fully understood, many observers see the increasing demands of the regulatory systems as one of the main drivers.

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Viability of GM Fungi Crucial to Malaria Control

IN THEIR REPORT “DEVELOPMENT OF TRANS-GENIC FUNGI THAT KILL HUMAN MALARIA PARASITES IN MOSQUITOES” (25 February, p. 1074), W. Fang *et al.* discuss a genetically modified (GM) fungus that removes *Plasmodium* parasites from infected mosquitoes and could thus reduce malaria transmission. Indeed, the transgene exclusively targets the causative agent of malignant malaria, *Plasmodium falciparum*. In light of emerging insecticide



resistance that may seriously undermine current malaria vector control efforts [including use of impregnated bednets and indoor residual spraying of insecticides (1, 2)], fungal biopesticides should be developed as viable and sustainable biological alternatives. However, the authors have not yet tested whether GM fungi remain as viable over time as non-GM fungi. This viability will determine the frequency with which these organisms need to be applied as a control tool and thus their eventual cost-effectiveness.

So far, no single, long-lasting delivery technology of fungal biopesticides for malaria control is available. The published GM technology will offer great opportunities to better understand and improve fungal properties, but we have not yet fully exploited the potential of nontransformed entomopathogenic fungi to reduce the burden of malaria.

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TECHNICAL COMMENT ABSTRACTS

Comment on “Calcareous Nannoplankton Response to Surface-Water Acidification Around Oceanic Anoxic Event 1a”

Samantha J. Gibbs, Stuart A. Robinson, Paul R. Bown, Tom Dunkley Jones, Jorijntje Henderiks

Erba *et al.* (Reports, 23 July 2010, p. 428) attributed calcareous nannofossil morphology and assemblage changes across Cretaceous Oceanic Anoxic Event 1a to the effects of surface ocean acidification. We argue that the quality of carbonate preservation in these sequences, the unsupported assumptions of the biotic response to acidity, and the absence of independent proxy estimates for ocean pH or atmospheric $p\text{CO}_2$ render this conclusion questionable. Full text at www.sciencemag.org/cgi/content/full/332/6026/175-b

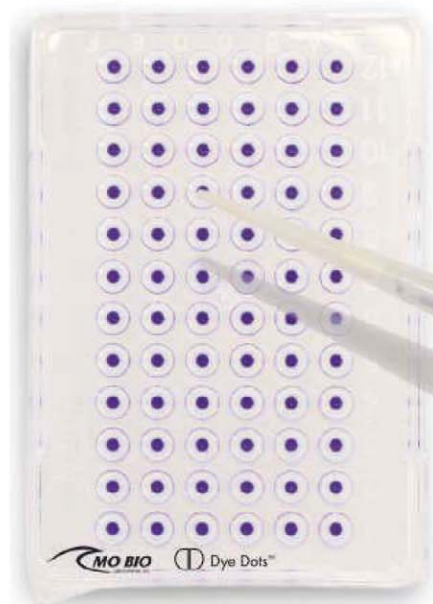
Response to Comment on “Calcareous Nannoplankton Response to Surface-Water Acidification Around Oceanic Anoxic Event 1a”

Elisabetta Erba, Cinzia Bottini, Helmut J. Weissert, Christina E. Keller

Gibbs *et al.* question our reconstruction of surface- and deep-water acidification around Oceanic Anoxic Event 1a. We answer their criticisms to better substantiate our arguments and original conclusions. Contrary to their suggestion, preservation cannot explain the nannofossil changes we documented, which trace perturbations in the photic zone, including a substantial increase in partial pressure of CO_2 ($p\text{CO}_2$) and an inferred decreased pH as derived from geochemical proxies.

Full text at www.sciencemag.org/cgi/content/full/332/6026/175-c

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HISTORY OF SCIENCE

A Séance for Science

Hanna Rose Shell

Marie Curie and her husband, Pierre, whose codiscovery of radium earned them a Nobel Prize in Physics in 1903, were a scientific couple made for storytellers. Madame Curie in particular seems almost to have been born a heroine: inspirational female scientist rises up from hard knocks, falls in love with perfect intellectual match, fends off the paparazzi, then endures multiple heartbreaks and the international rumor mill. She discovers radioactive elements and changes the world. The 1943 Hollywood biopic *Madame Curie*, based on Ève Curie's best-selling biography (*I*) of her mother, exemplifies this historical narrative and its emotional appeal. Its theatrical trailer enticed: "The love story of the most exciting

irony). The effects of Hiroshima and Nagasaki intensified growing fears about radioactivity worldwide. And yet even in the aftermath of the great nuclear catastrophes of the 20th and 21st centuries, the Curies retain their radiant glow as scientific and popular icons. More than half a century after the detonation of Fat Man, Lauren Redniss has used art to reconcile these two facets of radioactivity's emotional history through a restaging of Marie Curie's biography.

In *Radioactive: Marie and Pierre Curie: A Tale of Love and Fallout*, Redniss (who teaches at the Parsons School of Design)

Radioactive

Marie and Pierre Curie:
A Tale of Love and Fallout

by Lauren Redniss

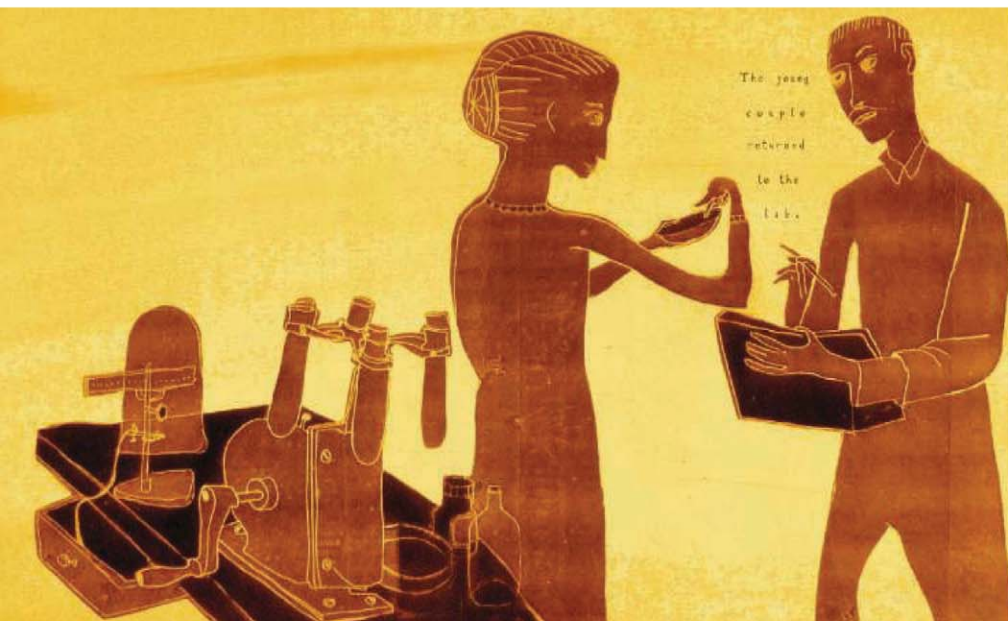
HarperCollins, New York,
2010. 208 pp. \$29.99.
ISBN 9780061351327.

in itself. One that, thanks to its luminescent cover, literally glows in the dark.

Interjections into the main story line (characters meet, fall in love, Nobel Prizes, fame, scandal) range from the quirkily amusing to the deadly serious. One is an upbeat list of assorted famous people, plants, and animals from Poland; another, a situational map of the Chernobyl exclusion zones. A particularly interesting series of these interjections proceeds from Oppenheimer's role as scientific director of the Man-

hattan Project to nuclear reactor-engineer Irving Lowe's 1943 "sacrificial mission" to try to get President Roosevelt to speed up U.S. efforts to develop an atomic weapon (complete with declassified excerpts from Lowe's FBI file) to a first-person testimonial by Sadae Kasaoka, who was a 13-year-old child in Hiroshima in 1945. However, the textural bridge into the series is gloriously tenuous: Marie's second child, Ève, was born in December 1904. "That same year ... Ella Oppenheimer, a pretty, slender painter with a congenitally disfigured right hand, and her husband, Julius, a lanky importer of textiles, also had a child. Their blue-eyed son would grow up to love poetry and literature, and to make his name as a physicist."

Artworks, the author's own, provide a matrix for these temporal leaps and sometimes-disjunctive histories. They are at the heart of the book's richness and the glue that binds it together. There is no page in *Radioactive* that is not an artistic work. The words always exist within a visual environment of color, texture, and patina. The placement and layout of text in relation to images often turn the words into design elements themselves. Redniss even designed the typeface, basing it on archival manuscript title pages and naming it after the Curies' preferred spiritualist medium. This is not design in any simple sense, but rather design as a means for creatively expressing, rather than narrating, history and information. For example, a short biography of physicist Paul Langevin—whose affair with Marie Curie overshadowed her second Nobel Prize (Chemistry, unshared, 1911) and made her the subject of international scandal and the talk of the French scientific community for years to come—takes on the literal shape of its subject. The margins of the text (on a right-hand page) outline a silhouette of his portrait, mirroring a hand-colored cyanotype by Redniss displayed on the opposite page.



woman of her day!—The drama of the man who shared her astonishing adventures!—The truth behind their strange journey into the unknown!"

Unbeknown to most moviegoers, just as the film was opening at theaters across the United States in November 1943, J. Robert Oppenheimer was hard at work developing radioactive plutonium-based nuclear weapons as part of the atomic bomb project at Los Alamos (in retrospect, something of a tragic

injects the often-told story of the Curies with a vibrant new energy. Over the course of nine chapters, one encounters a compendium of art, science, and passion. One does not read the book, one enters into it—an experience somewhere between walking into a cutting-edge museum exhibition and seeing into the mind of an artist's imaginings. The work, which draws heavily on Ève Curie's account among other publications, is a channeling of history. Its focus leaps from heartbreak to the chemical properties of polonium. Crafted around episodes already known well to both scientists and students of the history of science and technology, it is truly a thing

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Radioactive's imagery, far more than its words, creates an experience of entering into the author's own vision of history, her channeling of this tale of science and romance, discovery and betrayal. Readers will surely appreciate looking through the scattered scanned archival materials (photographs, maps, and letters); some are real gems. When these samples of other media work best, they trigger something like the feeling of rummaging around in an attic or archive. The musty smell takes over, and you really

know that you've found something good. Also very effective is the formerly classified document reproduced in the historical excursion to the Manhattan Project, wherein what is most important is the very fact that one's ability to read is obscured. Most striking, however, are Redniss's original artworks, the majority being cyanotype prints. These are ghostly Prussian-bluish photograms, produced through the same method long used by engineers and draftsmen to make blueprints and by children to turn plant specimens into

art projects. One can't smell the vapors of the reactions between ammonium and potassium compounds that create the images, as one would in an actual chemical darkroom, but one can feel their visual effects on almost every page. *Radioactive's* alchemical history exposes a new genre of science biography.

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10.1126/science.1204034

MEDICINE

A Call to Reorient Healthcare

Ronald L. Krall

Next Medicine offers Walter Bortz's remedy for what ails healthcare in the United States. In it, his seventh book, Bortz (a physician at the Stanford School of Medicine) assesses the state of the American healthcare system as he would a patient: listening to its symptoms, examining its signs, studying its performance, reaching a diagnosis, and prescribing a treatment.

What does he find? Bortz sees "current medicine" (his name for U.S. medicine as practiced today) as costing too much, too often harming people, unjust, corrupt, and inefficient. None of these five observations is new, but I find his perception that current medicine is "irrelevant" insightful.

Bortz uses "the term 'irrelevance' to signify a complete disconnect between Americans' health and our health care system." He spotlights a body of work that suggests that more than half of the illnesses we treat and more than half of our healthcare system's patient encounters result from modifiable behavior. Current medicine focuses on the tip of the iceberg that presents as "illness" while ignoring the behaviors that if modified or eliminated would prevent, delay, or ameliorate expressed illness.

The author believes this has happened because "[s]pecialization has become medicine's dominant agenda." It's the role model for medical students. It's what's rewarded by the compensation paradigm. It's the focus of nationally funded research. It's promulgated by the for-profit medical industry, especially pharmaceutical and medical device com-

panies, what Bortz calls the "disease cartel."

Bortz recalls the twin components of Greek medicine, Hygeia ("health preservation") and Panacea ("repair"). Through most of the history of medicine, Hygeia was dominant, but today Panacea reigns supreme. Bortz believes that "Panacea's elaborate repair capacities are brilliant, but its toolkit of surgery and pharmacy does not fully ensure that we humans will reach our full potential." Through this shift, medicine has come to concentrate on technology and drugs to diagnose and treat disease. The patient, the person, has been lost. Control and, more importantly, responsibility have been taken away from people, enabling behavior that is contradictory to their health. Thus current medicine has become irrelevant.

After reviewing medicine's current state and historical foundations, Bortz offers his diagnosis, "total body pain." He identifies the underlying cause of this systemic problem as "a mismatch between the basic forces of human biology and capitalism." The treatment he prescribes is nothing short of a revolution in the orientation of medicine, a shift from a focus on disease to one on health.

The author argues that it is time to turn to a "next medicine." Its first principle would be the recognition that medicine's mission is "to assert and assure human potential." The new medicine would entail practice that focuses on the whole person (not her or his parts) and that emphasizes individual responsibility. It would promote health literacy through efforts in schools and in communities, carried out by a new cadre of health educators. It

Next Medicine

The Science and Civics of Health

by Walter M. Bortz II

Oxford University Press, Oxford, 2011. 265 pp. \$34.95, £22.50. ISBN 9780195369687.

would incorporate findings from experiments in behavioral modification. Next medicine would encompass a reorientation back toward generalist physicians and "low tech" interventions as well as a reward system that pays for wellness rather than for treating disease.

Bortz would implement next medicine in a national healthcare system, involving a dozen or so regional health maintenance organizations that would be rewarded for superior outcomes. He proposes moving a substantial portion of current healthcare investment toward health education, behavior modification, and disease prevention. He claims that doing so will be cost-effective, yielding both great reductions in expenditures and improvements in health. In describing "health," Bortz draws from his research and practice in aging and the roles physical fitness has played in his own and others' lives. He believes we have the potential for far greater spans of life and quality of living than most people realize.

Perhaps the book's most controversial proposal is the recommendation to incentivize personal health behavior through risk-adjusted premiums: "The more disrespectful you are of your potential, the more you pay a logical risk adjustment." Bortz has clearly agonized over this approach, and he justifies it as the only way to marry the capitalism of healthcare with the need to change personal behavior.

Bortz thoroughly documents his diagnosis of current medicine. His digressions into the history of medicine and the profession, while interesting, make minimal contributions to his case. *Next Medicine* is short on exploration of the challenges facing any implementation of his prescription, but its case for a new strategic direction for medicine makes it worth reading.

10.1126/science.1204353

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Mekong Hydropower Development

R. Edward Grumbine^{1*} and Jianchu Xu²

The Mekong River is one of the world's last large rivers remaining mostly undammed. But China is constructing a series of eight hydropower projects on the upper Mekong. Although there are currently no dams across the mainstream channel (not including the tributaries) in the Lower Mekong Basin (LMB), nevertheless, in September 2010, the Lao People's Democratic Republic petitioned the Mekong River Commission (MRC) to begin the formal process of approving the first of 11 proposed dams across the lower Mekong (see the figure) (1). Although such a cascade would provide substantial power, it would likely reduce biodiversity and ecosystem service values of the LMB, while undercutting the livelihood and food security of millions of people. Decisions on this initial proposal expected over the coming months by the MRC countries may contribute to promoting high-impact hydropower development or to a movement toward integrated, transboundary river-basin management that could serve as a model for other rivers.

Biodiversity and Human Livelihood

Three factors lend Laos' petition particular importance. First, Mekong River Basin planning historically has proceeded without analyses, such as MRC is now performing, that integrate existing ecosystem and human livelihood vulnerabilities with projections of development of regional natural resources and potential climate-change impacts (2). Reliable data on LMB natural resources have been difficult to obtain because they have not been collected and government transparency is inadequate.

But this lack of integrated planning is not solely due to lack of knowledge; much is already known about the Mekong. The river flows through an area characterized by high poverty and low development. The poverty rate (earnings of less than U.S. \$1.25/day) averages 19%, 21% of the population do not have access to clean water, and some 30%

lack access to closed sanitation systems (3, 4). Projected impacts of climate change by 2050 range from low (e.g., reduced water availability), to moderate (e.g., increasing temperatures), to potentially high (e.g., decreasing food production and sea level rise in the Mekong Delta) (5).

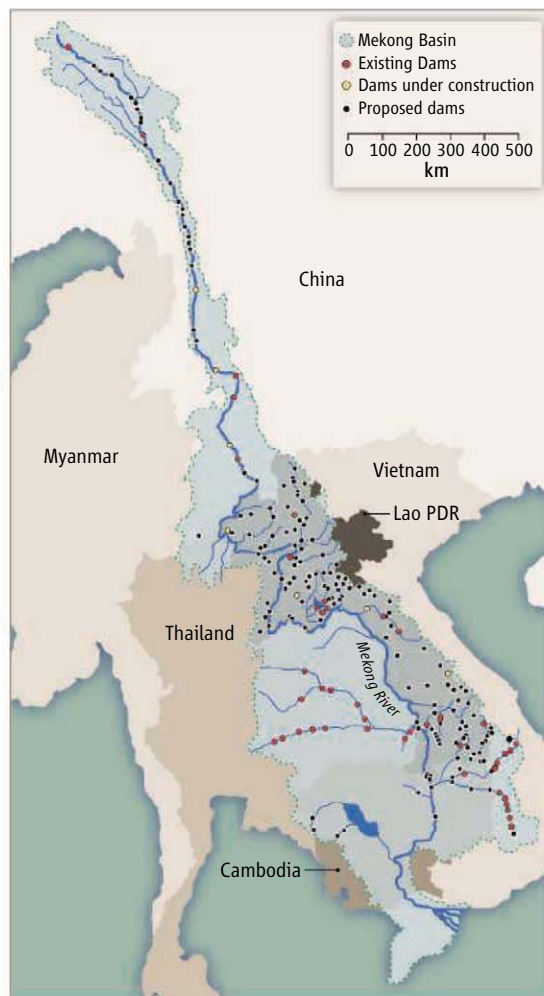
The second factor that bears on Laos' petition is MRC's release of the first-ever Strategic Environmental Assessment (SEA) of cumulative environmental and social impacts of all proposed mainstream dams (6). If built, the 11 dams would generate roughly 15,000 MW of power, projected to account for 8% of regional demand by 2025. Gross income

Pending decisions may unleash lower Mekong dam construction, despite management challenges and troubling environmental and social impacts.

from hydropower generation might total \$3.7 billion/year. Additional monies may be generated by increased regional trade and foreign direct investment, as power infrastructure is built and overall development proceeds. However, dam operators and investors would derive the most direct financial gains, because they would have exclusive rights to hydropower income during the first 25 years of dam operations. Nevertheless, Laos and Cambodia could gain annual income equivalent to about 18% and 4% of 2009 gross domestic product, respectively.

Environmental costs, however, would also be high. The SEA projects that dams would create direct costs—reduced fisheries, inundation of river bank gardens, and loss of nutrients for floodplain agriculture—equivalent to \$500 million/year. Ecological impacts would be severe. The Mekong ecosystem, which experiences seasonal hydrological pulses during monsoon, harbors the second-highest fish species diversity in the world, with many highly migratory populations (7). But dams would turn 55% of the lower mainstream into reservoirs with slow-moving water. Despite the migratory nature of Mekong fishes, only 3 of the proposed 11 dams incorporate fish ladders, and none of these designs are adequate for local species. Fifty to 75% of total river sediments would be trapped behind dams and prevented from moving downstream to nourish river primary productivity and floodplain farms (8).

These ecosystem impacts relate directly to loss of human livelihoods, for the Mekong is the most productive inland fishery in the world (9). With fish-dependent diets, the national populations of Cambodia and Laos would lose up to 30% of their annual protein intake. The SEA projects that roughly 2.1 million people would suffer direct and indirect losses to their livelihoods. Finding that the immensity of the risks was “beyond the current capacity of the LMB



Mekong Basin Hydropower. [Map courtesy of the Consultative Group on International Agricultural Research (CGIAR) Challenge Program on Water and Food; data from MRC and the Government of Laos PDR]

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region and its governments to address,” the SEA team recommended deferring all LMB mainstream dam building for 10 years.

The SEA’s assessment of current governmental capacities to manage the Mekong highlights the third factor influencing Laos’ petition. Others agree with the SEA that all LMB countries lack institutional capacities to manage transboundary natural resources (10). Yet international rivers require effective cooperative approaches if success is to be sustained over time. Exacerbating lack of governmental capacity is lack of funding to build needed management skills. In the past, much funding for river basin planning (and dam construction) came from multilateral development institutions (e.g., World Bank, International Monetary Fund, and Asian Development Bank). Now these agencies, with their commitment to some level of environmental and social impact assessment, are being replaced by investment from private and state-owned banks. These new investors, likely focused on profits, may not consider recommendations of the World Commission on Dams or other corporate social responsibility guidelines (11). Laos’ initial Xayaburi dam, for example, would be constructed using Thai bank capital, with the electricity bound for Thailand (12).

River Basin Planning for Sustainability

Efforts are under way in the Mekong to implement SEA recommendations. But such planning is predicated on governments’ support for a dam-building moratorium. While hydropower development is postponed, foundations can be laid for LMB planning that could also serve as a model for other Asian rivers. The first step would be to recognize political sensitivities involved with a shift from sovereign state-focused development to an integrated transboundary approach. Strengthening the MRC as a regional forum for discussing transboundary issues would help build trust that is essential to negotiate complex matters of resource use in a world of increasing scarcity, rising uncertainty, and declining resiliency (13). The South Asia Water Initiative Assessment is an existing forum that could serve as a useful guide.

A second step would be to focus institution building efforts on two arenas: transboundary knowledge and transgovernment parallel networks. Both would emphasize sharing benefits through the balanced provision of water and power, flood control, regional trade, and livelihood and food security (14). Such networks are being established by the Mekong Program on Water Environment and Resilience (M-POWER) and the MRC with

its efforts to create regional data systems for information sharing and flood control center, and an early warning system. A missing piece is evaluation of non-hydropower-based energy; there has been little country or region-wide assessment of future power needs that weighs alternatives beyond dams. Data sharing cannot be completely separated from transboundary government networks for long-term management of the Mekong. China, for example, has never joined the MRC. When China’s dam cascade is complete, it will have altered historical baseline low-flow levels of the Mekong (15).

Another essential step is enhancing transparency, public participation, and financial benefits for local peoples. This will likely remain a slow process in the Mekong, where there is a history of top-down government authority and people most affected by dams have not been part of decision-making processes (16). But existing water-planning frameworks where people are included from the earliest stages could be applied in the Mekong (17). Since 2007, Vietnam has been sharing hydropower revenues with poor rural households through a system of payments for environmental services. In 2 years, this scheme has generated \$4 million, roughly 90% of which has been returned to villagers for protecting local forests.

Multilateral support to foster knowledge and governmental networks in the Mekong basin remains essential. Even the wealthiest countries (Thailand, Vietnam, and China) do not have financial resources to address current environmental vulnerabilities and future risks. A full economic accounting of such costs has never been completed in the Mekong, but the Asian Development Bank is well positioned to spearhead this work. Such a report would be useful to multilateral funding agencies. Unlike multilateral banks, private dam investors are less likely in the short term to address environmental risks. But given private financing of future dams, investors must be encouraged to commit to environmental and socially responsible assessment and implementation of hydropower projects.

Adaptive Planning and Geopolitics

The MRC has been “an amputated river basin organization” [p. 61 of (18)], as upstream countries are not included, and there is little assessment of tributary dam developments. Today, all the pieces are in place to create an innovative management model that could be useful across the region; transboundary environmental, livelihood, water, and food security vulnerabilities on the Ganges-Brahmaputra-Meghna and Indus Rivers are similar

(19). Upstream, China’s dams are only partially built, but they will increasingly influence dry-season flows, sediment capture, and overall transboundary hydropower management. And Chinese companies are set to finance many of the proposed new LMB dams. In 2009, the U.S. Department of State announced funding for a Lower Mekong Initiative to build capacity for a range of environmental, education, and health projects in the LMB. In an era of rising resource demands, reduced environmental resiliencies, and ongoing climate-change impacts, international cooperation across multiple scales is in the best interests of all.

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ASTRONOMY

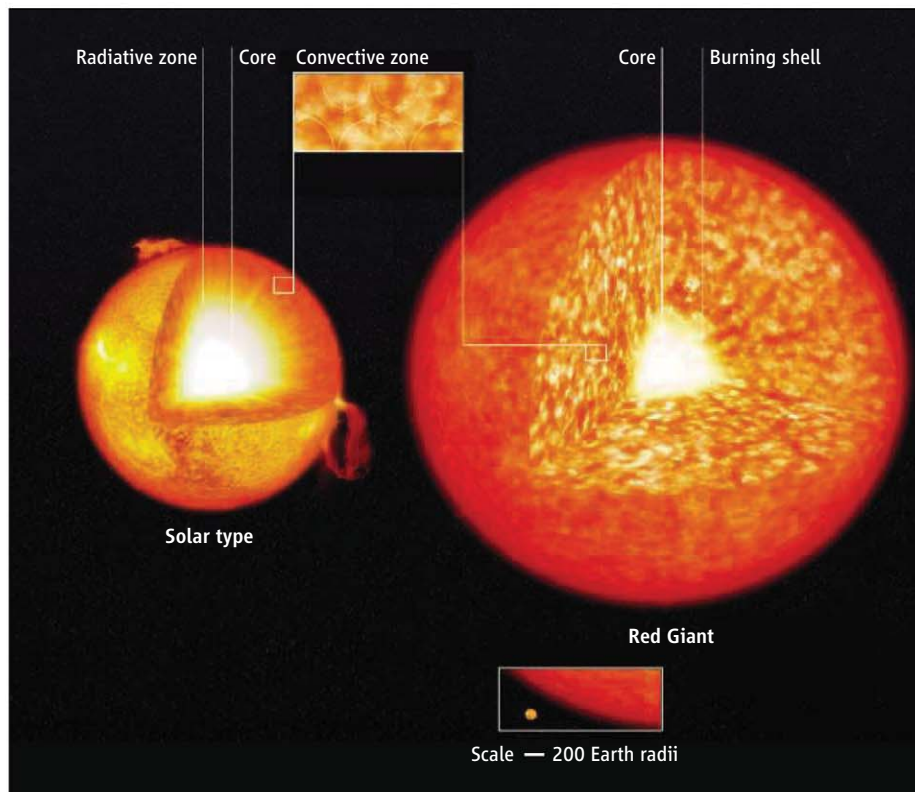
Shooting for the Stars

M. H. Montgomery^{1,2}

The primary objective of the Kepler mission is the discovery of Earth-like planets that are transiting their central stars. Its exquisite photometric precision also makes it the ideal instrument for measuring low-amplitude brightness variations in a broad range of stars. In a great many cases, these stellar variations represent modes of oscillation of the star, and their study can yield information on its interior structure. On page 213 of this issue, Chaplin *et al.* (1) make use of recent Kepler observations to examine the ensemble properties of more than 500 “Sun-like” stars. On page 205, Beck *et al.* (2) describe results for a red giant star. Finally, on page 216, Derekas *et al.* (3) present data on an exotic triple-star system with multiple eclipse components. Taken in combination, these latest results illustrate the power of the Kepler space telescope to probe the internal structure of distant stars.

The most familiar of the variable stars are the cepheid variables, the “Cepheids.” They have been known for hundreds of years and their large changes in brightness make them easy to observe. More fundamentally, the link between their period of oscillation and their intrinsic brightness—the “Leavitt Law” or the “Period-Luminosity relation”—has made them important rungs on the astronomical distance ladder (4).

Although other variable stars have difficulty competing with the Cepheids in terms of brightness, they easily outstrip the monoperiodic Cepheids in terms of the number of oscillation modes they possess. The champion of these is the Sun, whose pulsation modes induce luminosity variations of only a few parts per million but whose number of excited modes exceeds a million (5). The reason for this contrast is the completely different driving mechanisms at work. In the Cepheids’ case, the energy flowing outward through the envelope of the star is dammed up and released in a way that leads to an amplification of existing perturbations, so that small-amplitude oscillations are amplified to observable levels. In contrast, in the Sun the bubbling fluid motions in its convection zone



Star gazing. The convection zones of a solar-type star and a red giant. The outer convection zones in both types of stars are effective at driving pulsations.

shake the star as a whole, causing it to ring. Individual oscillation modes are damped and begin to decay after they are excited, but are continuously re-excited by the fluid motions in the convection zone, so that a balance is reached between driving and damping.

Stars whose pulsations are driven by this mechanism are termed “solarlike” pulsators, and while their amplitudes are small, they oscillate in many modes over a broad frequency range. This is fortunate because each pulsation mode samples a star’s interior differently, so the more pulsation modes that are observed, the tighter the constraints placed on the internal structure. These pulsations allow us to probe a star’s structure in much the same way that seismic waves on Earth allow us to sample Earth’s interior. Because of this analogy, the study of a star’s interior through its pulsation modes is termed asteroseismology and, for the special case of the Sun, helioseismology. Fortunately, solarlike pulsations are expected to be present in all stars having surface convection zones.

Observations with the Kepler space telescope are revealing details of the internal structure of distant stars.

This includes main sequence stars with masses less than about 2 solar masses, as well as red giants. Chaplin *et al.* use solarlike oscillations observed by Kepler to derive distributions of the radii and masses of 500 solar-type stars. They find that the distribution of radii matches theoretical expectations but that the mass distribution shows some important differences—the observed mass distribution is wider and shifted toward lower masses. If real, this is an important result and could lead to revisions in our understanding of the initial mass distribution with which stars form, as well as the mass-radius relation. It could also point to inadequacies in our description of convection in these stars.

Beck *et al.* report a second example of solarlike oscillations, providing evidence for the detection of gravity-mode period spacings in a red giant star. Gravity modes (g-modes) are oscillation modes in which the primary restoring force is buoyancy/gravity, in contrast to pressure modes (p-modes), in which the primary restoring force is pressure.

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The oscillations that are usually observed in these stars are p-mode pulsations, which sample the envelope but do not penetrate deeply into the core. The importance of the detection of the g-mode period spacings is that g-modes do penetrate deeply into the interior; thus, they contain information inaccessible with other techniques, such as the core chemical composition, angular momentum, or density structure. This is of prime importance because models of the red giant phase of stellar evolution are much less constrained than those of the prior main sequence phase. As a result of Kepler, we may well see revisions in our understanding of red giant structure and evolution.

Rather than reporting results of stellar pulsations, Derekas *et al.* use Kepler data to study the light variations from an exotic triple star system. The bright A component of this system is a red giant star, and the B and C components form a tight binary (BC). The

BC system orbits the A component. What makes this system the first of its kind is that it has two kinds of eclipses. First, stars B and C eclipse each other, and second, the BC pair and the A star eclipse each other. The eclipses provide constraints on the geometry of the system, which can then be used to test stellar models. In addition, continued observations will allow dynamical models of the evolution of the stellar orbits to be tested. In an ironic twist, the solarlike oscillations that are expected in component A (a red giant) are not detected; they are most likely suppressed by either the unusual orbital mechanics or some other aspect of this system.

Kepler is returning a treasure trove of information on the properties of stars. Although already impressive, these observations represent only the tip of the iceberg of what we will learn through continued observation with Kepler and other ground- and space-based telescopes. Future observations

will test our understanding of physical processes such as convection, rotation, chemical diffusion, mass loss, and magnetic fields in stars. Because stellar evolution calculations are our basic tools for modeling the properties of all stars, whether or not they pulsate, this work will enhance our understanding of the life cycles and properties of all stars. This in turn will allow us to better understand the evolution of our galaxy and the local universe.

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MOLECULAR BIOLOGY

Climbing in 190 Dimensions

Michael Yarus

Those who have looked at the night sky—not the dim remnant visible in cities, but the bright complexity seen in high, dark places—can appreciate the task assumed by Wochner *et al.* (1). On page 209, they describe the construction of an RNA enzyme (a template-dependent primed RNA polymerase) that emulates an ancient molecule that would have been crucial in the “RNA world,” believed to have predated DNA- and protein-based life. To find this enzyme, they searched vast molecular populations, holding potentially many, many more RNAs than the visible universe has stars.

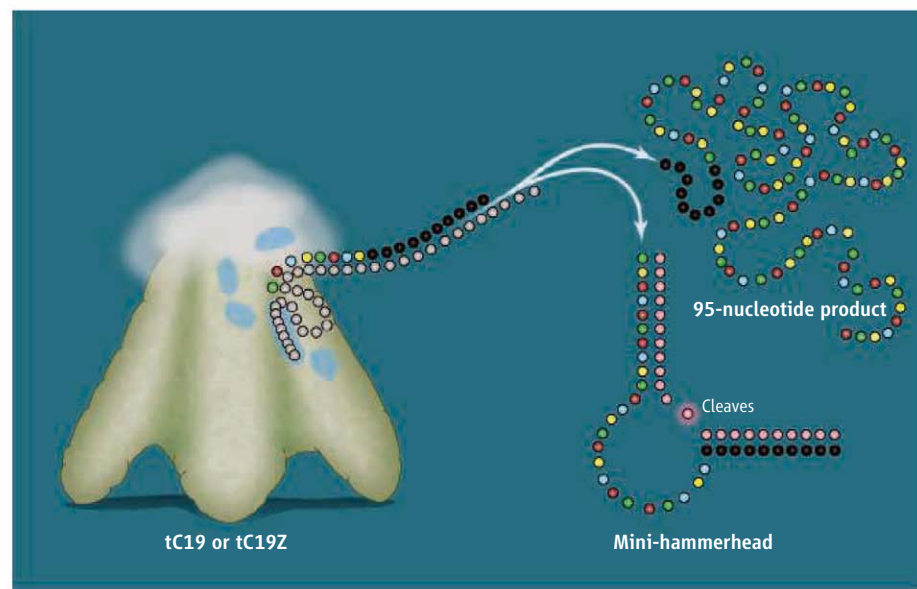
RNA's role in expression, and particularly in protein biosynthesis, has provided recent evidence of an ancient RNA world. One key part of this world, however, has remained lost—the ancestral RNA molecule that replicated everything, including itself. In a bid to find such a molecule, Wochner *et al.* began with the recently selected and well-studied R18 RNA (2). R18, however, can make templated RNAs only 14 nucleotides (3) or 20 nucleotides (4) long. These numbers frame the progress achieved by Wochner *et al.*, who

engineered RNA sequences that make RNAs up to 95 nucleotides long.

The crucial challenge was to explore an immense cosmos of sequence variants. R18 has 189 nucleotides, so there are 4^{189} or about

Creation of an improved RNA-copying ribozyme offers hope of realizing RNA-catalyzed self-replication.

10^{114} other sequences of the same length as the initial RNA. Adding a dimension for useful synthetic activity, optimizing evolutionary potential is a problem with 190 dimensions. One must seek a peak of RNA production in



Isolating an effective RNA polymerase. (Left) The tC19 or tC19Z ribozyme (8, 9), with ribozymic improvements (1) marked by blue patches. Template nucleotides are gray, primer is black, and products are four colors representing the four incorporated nucleotides. **(Right)** Selected RNAs produced long products (upper right, 95-mer) and an active mini-hammerhead (bottom).

this rough, complex landscape. To do that, Wochner *et al.* searched libraries containing a few $\times 10^7$ sequences. Such populations contain about 10^{-107} of the potentially relevant structures. This is an unimaginably small proportion of all such RNAs, but their search succeeded. How can this be? Even those sequences closely related to R18 RNA are too many to have been thoroughly mapped, despite much prior exploration. As one result, the sequences we know are almost certain to sit far from a major summit of optimality.

Part of the answer lies in the ingenuity and commitment of Wochner *et al.*, who created and wielded new methods. For example, using magnetic microbeads in about 750 μ l of oil emulsion (a technique termed “compartmentalized bead-tagging”), they transcribed thousands of clonal copies of a ribozyme, and then selected RNA products made to fluoresce (the researchers sorted the beads by the light they emitted). If you love apt and forceful molecular biology, you will love this virtuoso performance, which zeroed in on catalysts with lengthy products. More wizardry followed, including selection of a 5′ core sequence that bound the 5′ end of templates to secure them to the catalytic domain. Screening of experimenter-designed 5′ variants then maximized the ability to use longer primed templates. The result was polymerized products that were ≥ 91 nucleotides long on 0.035% of total primers, annealed to a particularly favorable repetitive tem-

plate sequence. Finally, the ability to make long products was generalized by recombining certain core mutations and screening for broad performance on several different primer/templates. The final ribozyme produced very accurate templating—just 13 substitutions and deletions in 2200 nucleotides of sequenced RNA products (99.4% correct).

To gather improved accuracy and chain extension into a single experiment, Wochner *et al.* challenged their final ribozyme, tC19Z (see the figure), to make the ribozyme strand of a mini-hammerhead (5). It did, lengthening 20% of primer beyond 24 nucleotides, the point at which the mini-hammerhead ribozyme domain is complete. The resulting self-cleaving ribozyme (in combination with a canonical substrate strand) was as effective as the ribozyme synthesized by normal means. For the first time, an RNA-synthesized RNA participated in a second, active, ribozyme.

An RNA polymerase capable of Darwinian evolution is now a large step closer. In the old days (say, 2007), we could template product RNAs that were only 8 to 11% as long as the polymerase. Now we are at 48%. One prediction of the 190-dimensional view is that there is likely to be a route leading up to longer RNA transcripts, if only we can find it. With luck, the very next slopes will take us to Darwinian altitudes, where we have not been before [but see (6) for another kind of replication]. However, we also ought to try using the bright tools developed and deployed so effec-

tively by Wochner *et al.* to climb some mole-hills. We have newly specific access to chain extension. Accordingly, we may be able to reopen the hunt for small RNA polymerases. It was abandoned because irrelevant activities dominated selections (2), not because small RNA-RNA polymerases are known, in any sense, to be impossible.

The RNA world likely ended with the invention of translation, allowing peptides to replace active RNAs. In studying this translational finale, it has become evident, in the wake of improved selections, that simple RNAs—as small as five nucleotides—carry out some translational reactions (7). Perhaps the time has come to use better-focused, more specific replicase searches on randomized populations of RNA, to look more deeply for a smaller templated phosphodiester transferase, which might have served as the gateway to the RNA world.

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MATERIALS SCIENCE

The Phase Behavior of Interfaces

Martin P. Harmer

Common metal or ceramic objects are made up of many bonded micrometer-scale single crystals, or grains. Their interfaces, called grain boundaries, play a decisive role in determining the properties and processing of almost all engineering materials. Because of their structural and chemical complexities, the description of grain boundaries has lacked a satisfactory conceptual framework, and grain boundaries tend to be viewed as disordered regions with kinetically trapped structures. Recent work has shown that grain boundaries can

be described as interface-stabilized phases (also called interphases or complexions) that are chemically and structurally distinct from any bulk phases (1–4). On page 206 of this issue, Baram *et al.* (5) extend our understanding of grain boundaries by examining interface structures that form between a gold particle in contact with metal oxide surfaces. They show that these nanoscale interface structures are equilibrium phases that obey thermodynamic rules analogous to those for bulk phases.

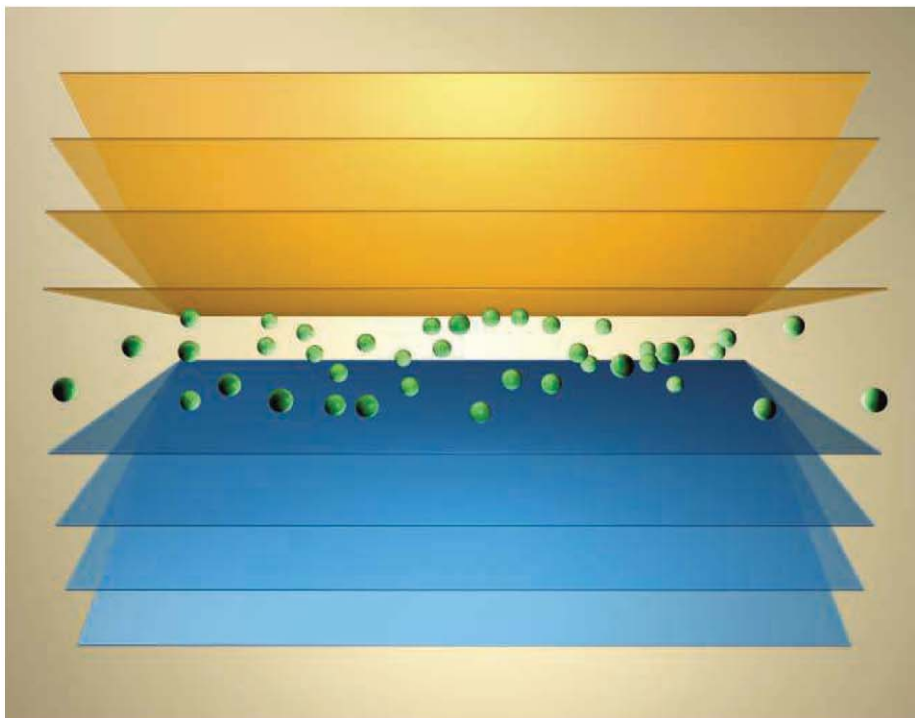
One example of a fundamental problem associated with grain boundaries, and why we need to understand their structure better, is abnormal grain growth. When a material is heated, atoms flow within the grain

Grain boundaries between crystals, which can control materials properties, can interconvert between well-defined equilibrium structures.

boundaries, and some grains grow preferentially abnormally large while others disappear. This can create defects and voids inside the material, which can weaken it. Another is embrittlement—impurities can diffuse and segregate into grain boundaries, which impedes the flow of atoms needed to make a material ductile. Conversely, deliberate incorporation of certain atomic elements into grain boundaries can dramatically increase a material’s resistance to failure at high temperatures from oxidation or deformation, increasing component lifetime.

The different types of structures that can form at grain boundaries have generally not been described as thermodynamic phases because they are not intrinsically stable as

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Defining boundaries. A schematic illustration shows one type of “complexion” phase that can form between different crystals within a material. The crystal planes that make up an interface are shown in blue and orange. The atoms that make up this complexion phase, shown in green, come from impurity, or dopant, elements that were introduced during the processing of the material. The complexion phase does not exist outside the confines of the grain boundary. It can be transformed into one of various other complexion states of matter in order to change the properties of the material. Baram *et al.* report that a nanofilm complexion phase between a gold particle and a metal oxide is a thermodynamically stable phase.

stand-alone phases, even though they are thermodynamically stabilized by the internal interfaces between the grains. The term “complexion” clarifies this important distinction in stability behavior between bulk phases, which may coincidentally reside on a grain boundary (see the figure) (1–3). Each complexion represents an equilibrium state of matter at a crystalline interface, which is neither amorphous nor crystalline. A progressive series of six complexion transitions has been proposed, whereby the discrete number of atomic layers and complexion layer thickness define the number of possible stable complexions. The six different states of complexions that have been distinguished thus far include pure (no layer between the grains), monolayer, bilayer, trilayer, nanofilms (e.g., Baram *et al.*), and wetting films (1).

Because complexions are equilibrium features, the way in which conditions and composition determine which complexion forms can be represented on a diagram analogous to bulk phase diagrams (1, 2, 4). The local boundary energy provides an extra degree of freedom, so boundaries with different complexions can coexist in thermodynamic equilibrium in the same material.

If different complexions have vastly different properties, which is likely to be the case, then the type of complexion that forms should greatly influence materials behavior.

Theoretically, grain boundary complexion phases and their associated transitions were predicted to exist by thermodynamic modeling (1–4, 6). The use of conventional high-resolution transmission electron microscopy (TEM) has directly confirmed the widespread existence of nanofilm complexions in many materials (7). Baram *et al.* used such an instrument to study the interfaces between gold droplets and a sapphire (aluminum oxide) surface that was partially coated with calcium-aluminum silicate (anorthite) glass beads. The anorthite formed an intergranular nanofilm that decreased the interfacial energy between the gold particle and the sapphire and that differed from a thicker wetting film.

Despite this demonstration by Baram *et al.* for phase behavior by nanofilm complexions, direct evidence for some of the thinner complexions (especially the bilayers and trilayers) is sparse and controversial. Such studies push the limits of resolution for conventional high-resolution TEM. Fortunately, that is no longer such a limitation

with the availability of the new generation of aberration-corrected scanning transmission electron microscopes of much higher atomic resolution (8).

Even if higher atomic resolution can be achieved, the difficulty of accessing internal interfaces makes the characterization of internal grain boundaries more challenging than studies of free-surface interfaces. Free surfaces exhibit layering transitions of adsorbates from monolayers to multilayers that can be formally treated as thermodynamically induced phase changes, which are often represented by surface phase diagrams.

Many exciting opportunities to explore and exploit grain boundary complexions are available given new techniques to observe and characterize them directly, along with the rapidly growing ability to perform realistic quantum-mechanical calculations and simulation methods to predict complexion structures and their transitions (9). Verifying the existence and stability range of grain boundary complexions in a wide variety of materials systems, such as metals, ceramics, semiconductors, natural materials, and biomaterials, is an important goal. Measuring and matching the properties with individual complexion types may enable the design of entirely new classes of materials with distinctive combinations of properties, as well as the optimization of the performance of existing materials in applications such as clean energy storage and conversion. The experiments reported by Baram *et al.* set the stage for the development of a rational and robust unifying scientific framework for the understanding of grain boundaries and provides a mechanism-driven approach to design grain boundaries and tailor materials properties (10).

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GENETICS

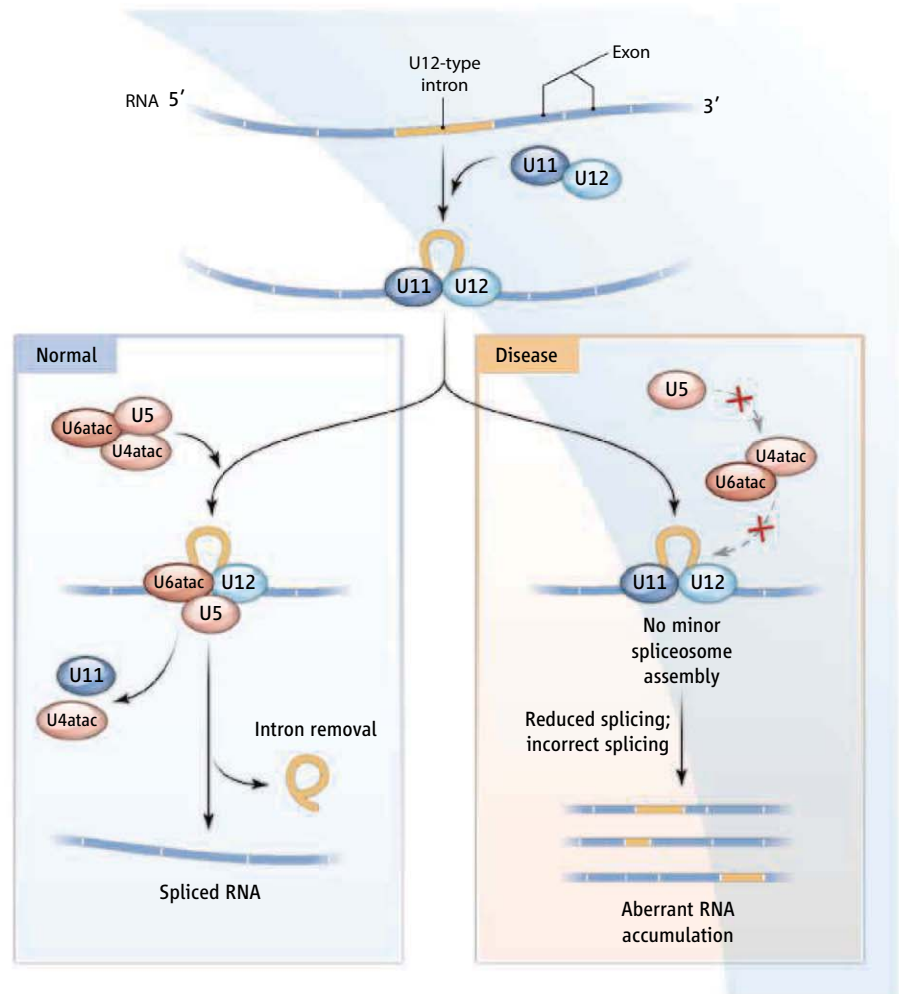
Minor Splicing, Disrupted

Heli K. J. Pessa and Mikko J. Frilander

The excision of noncoding intron sequences from primary transcripts, also called mRNA splicing, is a central step in eukaryotic gene expression. This process is necessary for producing functional mRNA molecules and thus for the viability of cells and organisms. Although many disease-associated mutations affect splicing factors or the sites where splicing occurs, only a few mutations have been identified in the core components of the spliceosome, an RNA-protein complex that carries out splicing. These mutations occur in the protein constituents of the spliceosome (1). On pages 238 and 240 of this issue, He *et al.* (2) and Edery *et al.* (3) identify human disease-causing mutations in a gene that encodes a small nuclear RNA (snRNA) constituent of the spliceosome. These mutations in the U4atac snRNA are associated with microcephalic osteodysplastic primordial dwarfism type I (MOPD I), also known as Taybi-Linder syndrome (TALS). This rare disorder causes severe intrauterine and postnatal growth retardation, multiple developmental defects in various organs, and death within 3 years after birth. The findings provide important genetic tools for diagnosing the disease and for counseling mutation carriers in affected families, but also have implications for understanding splicing diseases in general.

The spliceosome contains five small nuclear ribonucleoproteins (snRNPs), each consisting of an snRNA and specific proteins, and more than 100 non-snRNP proteins. U4atac is one of the snRNA components specific to the so called “minor” or U12-dependent spliceosome, which recognizes and removes a small subset of introns called U12-type introns (4). The gene encoding U4atac is present in the genome as a single copy, unlike the snRNA-encoding genes of the “major” U2-dependent spliceosome, in part explaining why diseases linked to snRNAs have not been observed before.

The majority of the MOPD I/TALS mutations in both studies cluster to a specific region in U4atac snRNA that forms a phylogenetically conserved stem-loop structure (3), which immediately suggests a mechanistic explanation for the underlying cause



Not so minor function. Defects in U12-dependent splicing are likely due to impaired assembly or activation of the U12-dependent spliceosome. Defective splicing of U12-type introns may lead to the accumulation of unspliced mRNAs; 700 to 800 human genes containing U12-type introns may be affected to varying degrees.

of the disease. The mutations most likely cause distortion of the conserved stem-loop (2, 3), preventing binding of a spliceosomal protein called 15.5 K that is normally associated with the structure (5). This protein, in turn, is an adaptor needed for ordered binding of four other proteins. One of these proteins (called hPrp31 or 61 K) is required for the association of U5 snRNP with the U4-U6 di-snRNP in the major U2-dependent spliceosome (6), and presumably also has an analogous function in the minor U4atac-U6atac di-snRNP. The U4atac-U6atac-U5 tri-snRNP is required for forming the catalytically active U12-dependent spliceosome (see the figure). Thus, the scenario emerging from these studies suggests that MOPD I/TALS mutations

cripple U12-dependent splicing by blocking the U4atac-U6atac-U5 tri-snRNP formation, while not necessarily affecting intron recognition by the U11-U12 snRNP. Alternatively, the essential unwinding of the U4atac-U6atac complex could also be affected (5). In either case, immature precursor mRNAs containing unspliced U12-type introns would accumulate in the nucleus, and would possibly be degraded by nuclear quality control mechanisms (7). Consistent with this scenario, He *et al.* and Edery *et al.* detected increased levels of unspliced U12-type introns in patient cells.

Approximately 700 to 800 genes in the human genome carry U12-type introns (8). These genes are loosely categorized as “information-processing genes,” including numer-

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ous genes essential to gene expression processes such as transcription, RNA processing, and translation, as well as DNA replication and repair, cytoskeletal organization, and numerous ion channels (8, 9). Genetic suppression experiments of He *et al.* suggest that U4atac mutations reduce U12-dependent splicing, and presumably the functional gene products, to less than 10%. Yet infants affected by the mutations are carried to term, are born (with multiple developmental defects but without gross malformations), and can live up to 3 years. How is this possible, given that the depletion of proteins specific to the U12-dependent spliceosome leads to cellular growth arrest (10)? One possibility is that the severity of the splicing defects is transcript-specific, as suggested by the data from patient cells in both the He *et al.* and Edery *et al.* studies, and from larvae of the

fly *Drosophila melanogaster*, which contain mutations in the U6atac snRNA (11). Alternatively, the activity and requirements for the U12-dependent spliceosome may vary in different tissues. Indeed, tissue specificity is seen in retinitis pigmentosa and spinal muscular atrophy, which are caused by mutations in ubiquitously expressed spliceosome components or in the snRNP assembly factor SMN (1), respectively.

The findings of He *et al.* and Edery *et al.* raise further questions about the mechanism and effects of the splicing defects. The next steps include biochemical characterization of patient cells and transcriptome analysis from multiple tissues, possibly using animal models, to understand the impact of the mutations and the connection to the disease phenotype. Additionally, cells carrying the mutations may serve as models to understand the mech-

anistic basis of similar diseases. Such investigations will hopefully also clarify the longstanding issue of whether the U12-dependent spliceosome is an outdated molecular relic that is being phased out or whether it has special regulatory functions in metazoan cells.

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MICROBIOLOGY

Rapid Insect Evolution by Symbiont Transfer

Francis M. Jiggins¹ and Gregory D. D. Hurst²

Microbiologists have long recognized the importance of horizontal gene transfer—the movement of genetic material between organisms—in adaptation. It is common for plasmids (rings of DNA that are separate from chromosomal DNA) and phage (viruses that infect bacteria) to move between bacterial species, and their movement is often accompanied by the transfer of traits, such as antibiotic resistance, that are of great adaptive importance. In contrast, in multicellular organisms, the mutational variation

upon which natural selection acts is generated independently within each species. It has recently become clear, however, that horizontal transfer of traits can play a major role in arthropod evolution. Studies of insects have detailed cases in which traits under selection are encoded by a bacterial symbiont. These microbes are commonly maternally inherited within species and therefore provide variation that is accessible for the process of natural selection. Like plasmids, however, symbiotic bacteria also occasionally move to a different host species. On page 254 of this issue, Himmler *et al.* (1) present just such a case study. It documents fast evolution after the introduction of a new symbiont into a population of

A symbiotic bacteria dramatically increases reproduction and survival in a common insect pest.

the whitefly *Bemisia tabaci*. They detail the spread of a *Rickettsia* bacterial symbiont that, in just 6 years, dramatically increased the survival and fecundity of its host in the southwestern United States.

The majority of insect species host bacterial endosymbionts that live within their cells and are vertically transmitted from parent to offspring (2). Some of these endosymbionts are mutualists that are essential for the survival of the insects, and these can have stable associations with host lineages that last for millions of years (3). Less well understood are the diverse array of “secondary” endosymbionts, which are not essential for the survival of their hosts and often only infect

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Secondary symbiont interactions with insects. (A) In pea aphids, symbionts affect parasitoid resistance, heat tolerance, host plant range, and color. (B) Rove beetles of the genus *Paederus* are defended against spider predation by the toxin pederin, produced by a *Pseudomonas* symbiont. (C) *Drosophila* is protected

against a range of RNA viruses by *Wolbachia* bacteria. (D) Whitefly fitness is enhanced by the presence of *Rickettsia*, which also distort the infected population's sex ratio toward the production of daughters. (E) The butterfly *Eurema hecabe* carries a *Wolbachia* that causes genetically male hosts to develop as females.

certain populations or individuals. There is a growing body of evidence that many of these symbionts represent an “accessory genome” of nonessential genes that can provide benefits, such as resistance to natural enemies (see the figure). Himler *et al.* show that these benefits can be considerable: Whiteflies infected with *Rickettsia* bacteria produced offspring at nearly twice the rate of individuals lacking the infection, and a higher proportion of the offspring survived to adulthood. The inherited *Rickettsia* roughly doubled the fitness of its host in laboratory assays, although the mechanism underlying the increased fitness has yet to be elucidated.

Perhaps the most remarkable observation of Himler *et al.* is the speed with which the *Rickettsia* spread through the population—just 1% of whiteflies were infected in 2000, compared with 97% in 2006. Reports from other species suggest that such rapid invasions may be commonplace in insects. A strain of the bacteria *Wolbachia*, for instance, swept through populations of *Drosophila simulans* in California in just 3 years (4). The rapid spread of this symbiont was predominantly the result of the bacterium causing cytoplasmic incompatibility (the sperm and egg could not form viable offspring), but it also resulted in increased resistance of the host population to viral infection (5). More recently, researchers reported that a *Spiroplasma* bacterium that protects its host against parasitic nematode worms has invaded populations of *Drosophila neotestacea* since the 1980s (6). Beyond these case studies, there are good reasons to believe that adaptation associated with symbiont-encoded traits will commonly be more rapid than adaptation based on mutations in existing insect genes. After lateral transfer, symbionts arrive in a novel host species as a complete genetic package, which has evolved over time to encode complex traits such as natural enemy resistance. Having been selected to encode a trait in their previous host, they will often encode this trait directly in their new host. These symbiont transfers therefore represent “macromutations” that may have a higher selective advantage than “normal” mutations.

Despite the benefits of infection, the relationship between whiteflies and *Rickettsia* is not as benign as it might at first appear. Inherited symbionts have commonly been classified into those that are “nice”—providing benefit to their host—and those that alter host reproduction and spread in the fashion of selfish genetic elements. Being maternally inherited, these microbes have an evolutionary interest only in the production and survival of female hosts; consequently, they have evolved

a variety of traits through which they promote the production and survival of daughters (7). Himler *et al.* found that, in addition to increasing the survival and reproductive success of infected whiteflies, the *Rickettsia* bacteria also caused their hosts to increase the proportion of their offspring that were female. The symbiont is therefore simultaneously acting as a beneficial partner of its female host and as a reproductive parasite, distorting the sex ratio away from that normally produced by the insect. Researchers have found evidence of similar “Jekyll and Hyde” consequences of *Wolbachia* symbiosis in flies; the bacteria both protect their host from a range of RNA viruses and manipulate host reproduction through cytoplasmic incompatibility (5, 8, 9).

Secondary symbionts not only are changing our view of insect evolution but also have important economic and medical implications. Past work has shown that symbionts can alter the species of plants that aphids feed on, potentially changing the number of crops the insects affect (10). Symbionts also alter the rate at which insects can transmit disease. In some cases, they decrease transmission (*Aedes aegypti* mosquitoes infected with *Wolbachia* are less likely to transmit dengue) (11); in others, they increase it (whiteflies infected with *Hamiltonella* bacteria are more likely to

transmit tomato yellow leaf curl virus) (12). The B biotype of *B. tabaci* studied by Himler *et al.* is an important crop pest that spread throughout the world in recent decades (13). The *Rickettsia* may cause *B. tabaci* damage to increase for two reasons. First, the infection directly benefits the *B. tabaci* individual that carries it and thus increases their rate of reproduction. Second, the female-biased sex ratio produced by the *Rickettsia* will increase the intrinsic rate of increase of its host species. It is now clear that symbionts can be both allied to and antagonistic to human interests. Either way, they will be a key feature of agricultural and vector entomology in years to come.

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IMMUNOLOGY

Eosinophils Forestall Obesity

Rick M. Maizels and Judith E. Allen

Eosinophils in adipose tissue maintain metabolic homeostasis by controlling macrophage activity.

Most human body fat is stored in adipose tissue, and under healthy conditions, it provides a balanced exchange of triglycerides in response to energy demands. But adipose tissue is also the cardinal locus of the metabolic syndrome, whose hallmarks include the accumulation of abdominal fat and insulin resistance, with cardiovascular consequences. Adipose tissue is dynamically linked with the immune system; indeed, the activity of macrophage cells has a key role in progression to obesity (1–4). On page 243 of this issue, Wu *et al.* (5) show that eosinophils, an immune cell typically associated with allergy and worm infection, regulate the macrophage activation state

in mammalian adipose tissue and may have an important role in metabolic homeostasis.

The type of macrophage activated in obese and nonobese individuals differs sharply (3). As a person's body fat increases, the number of macrophages embedded in adipose tissue rises (6). These “classically activated” or “M1” macrophages (CAMs) produce proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) that act systemically and affect metabolism by decreasing the sensitivity of other cells to insulin (7). Adipose tissue itself releases a hormone into the circulation called adiponectin that regulates glucose and fatty acid metabolism. However, in obesity, adiponectin production drops and macrophages produce resistin, which acts systemically to increase insulin resistance and glucose intolerance. This can lead to diabetes.

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Lean individuals have fewer macrophages per gram of body fat, which are in an “alternatively activated” or “M2” state (AAMs) (8). This state is induced by interleukin-4 (IL-4) or IL-13, cytokines typically associated with the arm of the immune system that causes allergy but also protects against infection with worms (9). Downstream of the IL-4 receptor α (IL-4R α), through which both IL-4 and IL-13 signal, is the nuclear hormone receptor peroxisome proliferator-activated receptor γ (PPAR γ) that, when activated by appropriate lipids, inhibits the expression of genes that promote inflammation and protects against insulin resistance (10, 11).

How does healthy adipose tissue induce and maintain AAMs, and what goes awry in obesity? Because macrophages require IL-4 or IL-13 to develop into AAMs, Wu *et al.* searched adipose tissue for the source of these cytokines using mice genetically engineered to express a fluorescent protein at the

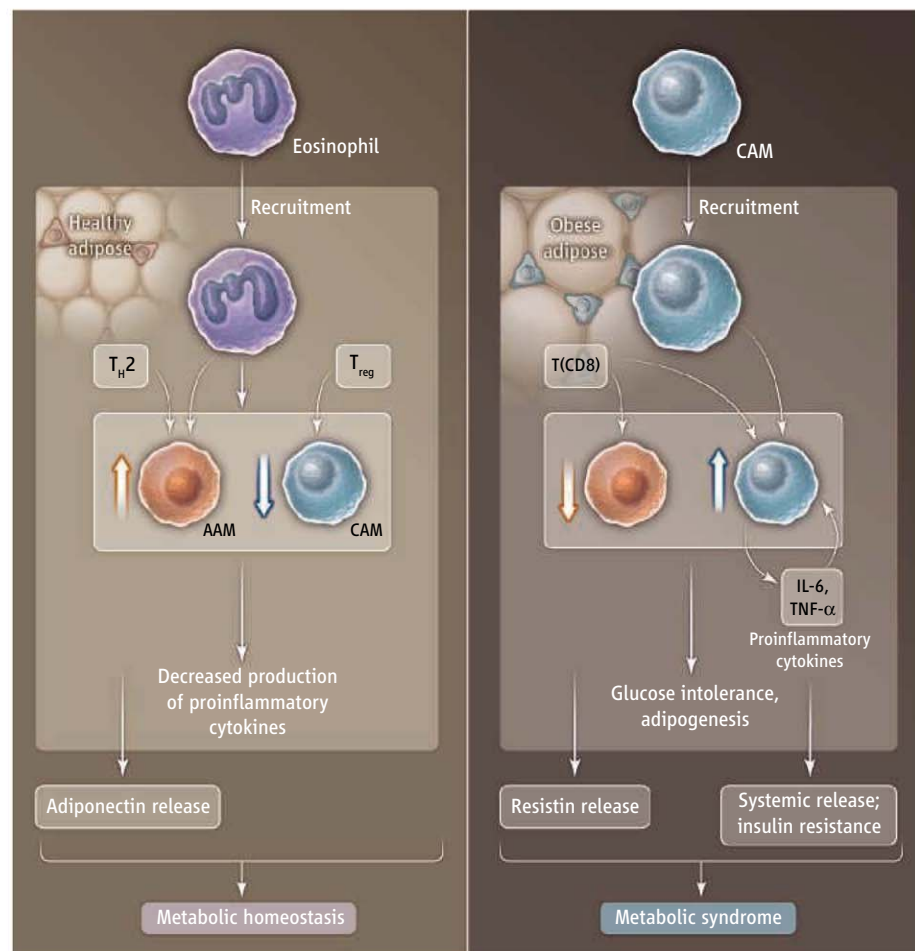
Il4 gene locus. They found the major source to be eosinophils and confirmed this in mice with an abundance or lack of these cells. In the former case, mice had less adipose mass and were more responsive to glucose, whereas animals lacking eosinophils became more obese and glucose resistant. The number of AAMs neatly tracked the eosinophil status of the mouse and corresponded inversely with susceptibility to obesity.

There are numerous potential triggers for the switch to CAMs in obesity. These stimuli in adipose tissue include dietary saturated fatty acids (12), ceramides (from fatty acid degradation) (13), and metabolic “danger signals” indicative of hypoxia and necrotic cell death (14). Activated lymphocytes in adipose tissue may also promote the switch. Whereas regulatory T cells dampen inflammation and obesity (15), cytotoxic T cells (CD8 subtype) can prompt adverse metabolic changes in adipose tissue (16). It is also possible that in the

absence of eosinophils in adipose tissue, IL-4 and IL-13 concentrations are too low to counter the effects of infiltrating T cells that produce IL-6 and TNF- α , which promote CAM development. Hence, insufficient AAM stimulation could prompt a metabolic disorder, even in individuals with healthy diets.

Wu *et al.* have now established that eosinophils promote adipose tissue AAMs, which in turn improve control of glucose metabolism. The authors studied healthy mice that were infection-free and thus had minimal lymphocyte activation. Lymphocytes, which are central to adaptive immunity, also regulate the recruitment and activation of leukocytes in fat tissue. T_H2 cells, which become activated in the adaptive immune response (to parasitic worms), produce IL-4 and might become its major source in adipose tissue during an active immune response and supplement or supplant the role of eosinophils in vivo. Because helminth infections frequently drive a sharp increase in the number of AAMs (9), they may directly reduce the risk of high-fat-driven CAM activation.

This raises the question of whether increased numbers of eosinophils or T_H2 cells, perhaps via worm infection, can reverse inflammatory damage and restore glucose control. When Wu *et al.* infected mice on a high-fat diet with the gastrointestinal nematode *Nippostrongylus brasiliensis*, their susceptibility to glucose intolerance, and their overall adipose tissue mass, declined. Moreover, a single infectious episode of up to 8 days caused sustained eosinophil presence in adipose tissue and protected from obesity. These findings provide an intriguing new dimension to the commonly held “hygiene hypothesis”: Can the rising incidence of metabolic syndrome, like the prevalence of allergies and autoimmunity, be exacerbated by the absence of certain parasites with which we have evolved?

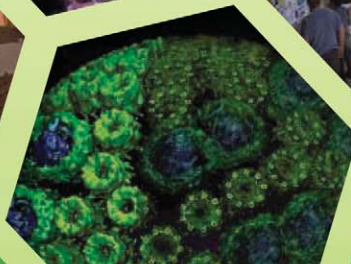
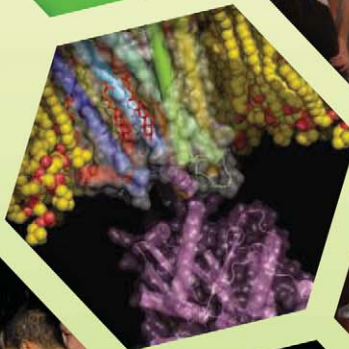
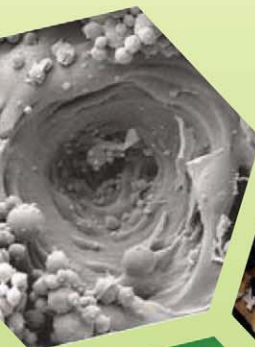


Immune cells in fat. (Left) In healthy adipose tissue, eosinophils produce IL-4, which sustains alternatively activated macrophages (AAMs). Classical activation of macrophages (CAMs) is suppressed by regulatory T cells (T_{reg}). Helminth infection may boost AAMs by expanding eosinophils, helper T cells, or both. **(Right)** In metabolic syndrome, high numbers of macrophages in adipose tissue are recruited and activated by T cells (CD8) or by inflammatory cytokines released from resident CAMs. Cytokines entering the circulation can cause insulin resistance. Adipose tissue releases resistin and free fatty acids into the circulation.

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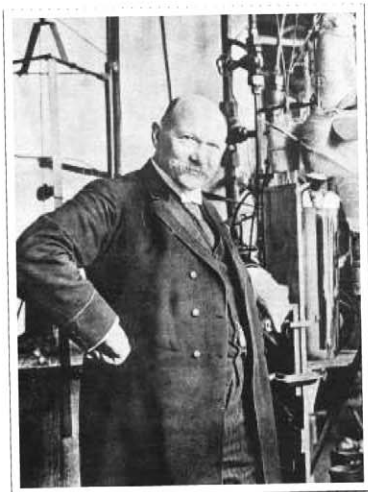
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INTRODUCTION

Happy 100th, Superconductivity!

Working in his cryogenics laboratory at Leiden University in April 1911, Heike Kamerlingh Onnes noted that the resistivity of the metal mercury appeared to drop to zero as the temperature in the cryostat was decreased to 4.2 K. He recognized that the effect was real and not due to a faulty electrical contact or some other experimental artifact, and found that other metals placed in the cryostat exhibited similar behavior. Superconductivity research was born.

It took nearly half a century before theorists formulated a comprehensive microscopic description of superconductivity. The picture involved electrons teaming up in pairs and colluding with distortions in the ionic lattice of the crystal so that the electrons could propagate through the lattice without any resistance.

This Bardeen-Cooper-Schrieffer, or BCS, mechanism (named after its inventors) gives a fine description of “classical” superconductors. After the mechanism was formulated, however, researchers began discovering exotic superconductors—including the heavy-fermion systems that came on the scene in the 1970s and the high-temperature superconducting cuprates that appeared in the 1980s—that defied such a simple explanation. The microscopic mechanisms of such unconventional superconductors are still hotly debated today.

Two Reviews in this special issue look at exotic superconductors—one new, the other not so new. On p. 196, Norman discusses the theoretical work that has developed over the past four decades to try to describe how unconventional superconductivity arises in the cuprates and heavy-fermion systems. Illustrating that such a mature field can still generate surprises and exotica, Wang and Lee (p. 200) discuss superconductivity in iron-based materials, a finding that has opened up an “iron age of superconductivity.” Since they were found to be superconductors less than half a decade ago, these materials have generated much interest in trying to fathom how magnetism and superconductivity, usually each other’s arch-nemeses, actually collude to form a superconducting state.

Meanwhile, as a pair of News stories makes clear, superconductivity and the theory behind it have enriched basic science in ways that nobody could have foreseen. Adrian Cho (p. 190) surveys how, over the past half-century, physicists have applied the BCS model of particle pairing to puzzles as diverse as the unexpected stability of certain nuclei, hiccups in the spin of neutron stars, and the bizarre behavior of liquid helium and supercold atomic gases. It has even helped inspire the discovery of new subatomic particles. Robert F. Service (p. 193) describes how condensed-matter physicists may be about to beat particle physicists at their own game. In experiments with superconductors, several teams of researchers are searching for so-called Majorana fermions: theoretically predicted particles that are their own antiparticles. Some now think they are on the brink of a discovery that could open a new window into quantum mechanics.

A century on, for more reasons than ever before, physicists and materials scientists remain enthralled with the observation of disappearing electrical resistance.

— JELENA STAJIC, ROBERT COONTZ, IAN OSBORNE

Superconductivity

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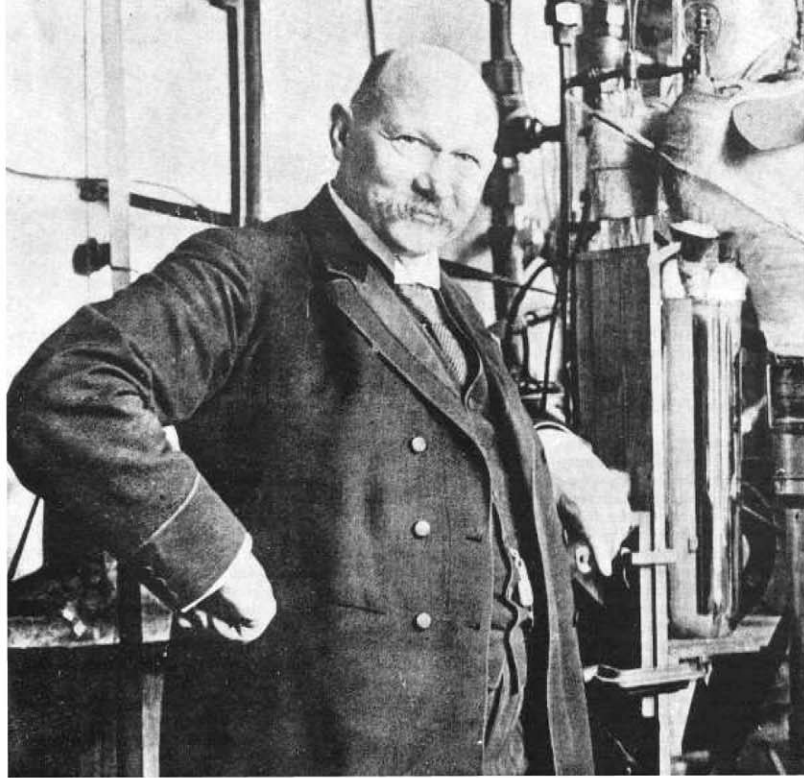
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Science



Chillin'. Heike Kamerlingh Onnes liquefied helium and discovered superconductivity.

NEWS

Superconductivity's Smorgasbord Of Insights: A Movable Feast

Resistance-free electric currents and their eventual explanation have influenced thinking far beyond solid state physics

ON 8 APRIL 1911, PHYSICIST HEIKE Kamerlingh Onnes scrawled in a notebook misdated 1910, "Mercury practically zero." Lost in a page of text, that cryptic phrase marks perhaps the most important discovery in the physics of materials. Kamerlingh Onnes meant that when he and his team at Leiden University in the Netherlands cooled a sample of mercury to within 3° of absolute zero, or 3 kelvin, its electrical resistance vanished so that current flowed through the metal with no energy to push it. Kamerlingh Onnes had discovered superconductivity.

The terse note gives no clue that the researcher had discovered one of the most bizarre tricks of nature. Physicists struggled for nearly 50 years to explain the phenomenon. When they finally did, the resulting theory would prove to be far more than just the explanation for one weird property of some metals. "It's much bigger than that, much bigger," says Frank Wilczek, a particle physicist at the Massachusetts Institute of Technology (MIT) in Cambridge who shared the 2004 Nobel Prize in physics.

Physicists have applied the theory of superconductivity directly to nuclear matter,

liquid helium, and ultracold atomic gases. Historically, insights from superconductivity convinced theorists of the importance of symmetries and the ways in which a physical system can muddle or "break" them. The concept of "spontaneous symmetry breaking" now undergirds theory in many fields, especially particle physics. "It was not a way that people were thinking, certainly not in elementary particle physics," says Gordon Baym, a theorist at the University of Illinois, Urbana-Champaign. Superconductivity, he says, "changed the way people thought in different fields."

That's not bad for an advance whose discoverer recorded it without so much as an exclamation point. Why did Kamerlingh Onnes make so little fuss over his observation? The short answer is that he was expecting the resistance of mercury to go to zero. (Technically, a material's intrinsic ability to impede current is its "resistivity"; a sample's "resistance" depends on its size.) But he expected that for the wrong reasons, and at first he overlooked the all-important way mercury's resistance dropped to zero.

A few years earlier, Kamerlingh Onnes

had embraced the opposite idea: that a metal at zero temperature would have *infinite* resistance. In 1902, British physicist William Thomson, a.k.a. Lord Kelvin, argued that at very low temperatures, the electrons in a metal would freeze to the ions in the material. So a metal's resistance should at first fall with temperature but then bottom out and climb again. In 1906, Kamerlingh Onnes cooled gold to 14 kelvin with liquid hydrogen. "A minimum of resistance seems not to be far off," he reported to the Royal Netherlands Academy of Arts and Sciences.

Then he proved himself wrong. In 1910, Kamerlingh Onnes used liquid helium, which he had achieved 2 years earlier, to measure the resistance of platinum down to 1 kelvin. It did not climb but simply leveled off. Data for gold suggested that the eventual level depended on purity. Kamerlingh Onnes realized that those data jibed with the ideas of German theorist Paul Drude, who argued that the electrons in a metal form a gas and ping off the vibrating ions but not one another. If so, Kamerlingh Onnes theorized, a pure metal's resistance should vanish at zero temperature as its ions stop vibrating.

To prove it, he needed a pure metal. Mercury was the obvious choice; at room temperature it's a liquid that can be distilled. But Kamerlingh Onnes predicted that mercury's resistance should fall gradually. In experiments in May and October it abruptly vanished at 4.2 kelvin. "His model couldn't make sense of this jump," says Dirk van Delft, a physicist and director of the Boerhaave Museum in Leiden. How does a metal's resistance just disappear?

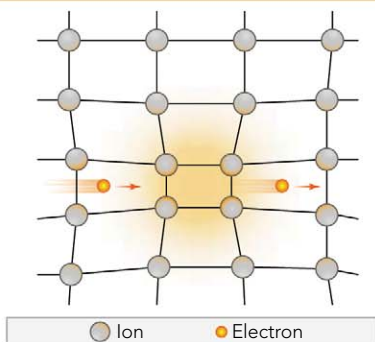
The hardest problem in solids

That mystery would stump the likes of Werner Heisenberg, Albert Einstein, and Richard Feynman. At the same time physicists explained myriad other properties of solids using quantum theory invented in the 1920s. "Superconductivity was driving people crazy," says N. David Mermin, a theorist at Cornell University. "It was the one thing that quantum mechanics didn't seem to clear up."

Whereas Drude had treated the electrons like marbles, physicists realized that they should be described by quantum waves that ripple with fixed energy and momentum. Also, electrons act like tiny tops that spin with angular momentum equal to half a fundamental measure called Planck's constant. Particles

CREDIT: COLLECTION KAMERLINGH ONNES LABORATORIUM

Conventional Superconductivity



Pulling together. Bardeen, Cooper, and Schrieffer (photo, *left to right*) explained that in a metal, one electron can pair with another by distorting the lattice of ions.



with $1/2$, $3/2$, $5/2$... units of angular momentum are known collectively as “fermions,” and a law of nature forbids two identical fermions from occupying the same quantum state.

So in a metal, the electrons stack into the lowest energy states. In an abstract three-dimensional “momentum space,” they fill a volume called the “Fermi sea.” Only the states near the surface aren’t crammed full and effectively clogged, so only they contribute to the flow of electrons. At the same time, the solid’s ions form an array known as a “lattice” whose vibrations are themselves quantum waves called “phonons.” Such theory explained many of the thermal, electrical, magnetic, and optical properties of solids. But not superconductivity.

Then in 1955, John Bardeen of the University of Illinois, Urbana-Champaign, set graduate student Robert Schrieffer and postdoc Leon Cooper on the problem. “I wasn’t totally aware of the number of people who had failed, but you can’t overestimate the arrogance of a young theoretical physicist,” says Cooper, now at Brown University. “If you’re not arrogant at some level, you can’t be a theoretical physicist.” Within 2 years the trio would show that superconductivity arises when the electrons in a metal form pairs that cannot be deflected without severing their bonds. That takes energy that’s not available at low temperatures, so the pairs glide unimpeded.

The “BCS theory” came together in a sequence of keen insights. In 1950, experimenters found that the precise temperature at which mercury becomes a superconductor depends on the isotope of mercury and hence the mass of ions in it, suggesting the ions play a key role. In 1955, Bardeen’s postdoc David

Pines had shown that in a metal, two electrons, which ordinarily repel each other, could in fact attract each other by tugging on the ions and creating phonons that draw them together—much as two people lying on a waterbed create dips that draw them together (see diagram).

A year later, Cooper showed that the feeble pull could bind two electrons surfing on opposite sides of the Fermi sea. That wasn’t obvious, Mermin says, because in empty space two particles must pull with a certain strength to pair. Cooper showed that the mere presence of the other electrons in the Fermi sea lowered the binding threshold for the two surfers to zero. “You can form pairs no matter how weak the interaction. That was Cooper’s great insight,” Mermin says.

Explaining how *all* of the electrons in the Fermi sea could pair took more doing. The theorists labored for months to find a quantum state that would achieve that while meeting the restriction that the electrons avoid each other. In January 1957, Schrieffer guessed the state’s mathematical form—while riding the New York City subway. “For what Bob did, there was no logical progression,” says Pines, now at the University of California, Davis. “It was a true ‘Aha!’ moment.”

The theorists now had a rigorous theory. At low temperatures, the electrons in some metals form a macroscopic quantum wave of intertwined pairs. The theory immediately yielded precise calculations and in 1972 earned Bardeen, Cooper, and Schrieffer a Nobel Prize.

Superconductivity still holds some mystery. In 1986, physicists discovered compounds of oxygen and copper that are superconducting at temperature up to 138 kelvin. Most physicists agree that phonons pull too

weakly to explain that. But nobody doubts that in such “high-temperature superconductors” electrons pair, Pines says.

Pairs, pairs everywhere

The BCS model was more than a one-trick pony. Bardeen, Cooper, and Schrieffer had based it on just two assumptions: that the particles are fermions and that they attract each other. So “it was obvious to all of us” that the theory would apply to other particles interacting through different forces, Cooper says.

First came applications to atomic nuclei. In the summer of 1957, before the BCS theory was published, Pines visited the University of Copenhagen. There he, Aage Bohr (Niels Bohr’s son), and Ben Mottelson found they could explain long-standing puzzles, such as why nuclei with an even number of protons and even number of neutrons are particularly tightly bound. The protons and neutrons, also fermions, independently pair. The work helped Aage Bohr and Mottelson win shares of a Nobel Prize in 1975.

In 1959, Russian theorist Arkady Migdal predicted that a stellar corpse known as a neutron star—a ball of neutrons bound by its gravity—should contain pairs of neutrons that form a free-flowing “superfluid.” The model can explain why rapidly spinning, energy-emitting neutron stars called pulsars sometimes suddenly speed up as swirling whirlpools migrate en masse out of the star’s superfluid interior. A pulsar then slows down again only gradually over months, says Illinois’s Baym. “That’s very hard to do without a superfluid,” he says.

The BCS theory may apply to neutron stars in a weirder way. Since the early 1970s,

Superconductivity

physicists have known that protons and neutrons consist of particles called quarks, also fermions. In the ultradense heart of a neutron star, the neutrons may dissolve into these constituents, which would then be free to pair. Scientists don't yet have experimental evidence for such pairing, says MIT's Wilczek. Still, he says, "I think the theory is pretty firm. It's just a question of whether the density gets high enough."

Closer to home, physicists have applied the BCS theory to liquid helium. In the 1960s, theorists predicted that magnetic forces might cause atoms in liquid helium—specifically, the isotope helium-3, which is a fermion—to pair and flow without resistance at temperatures as high as 80 thousandths of a kelvin (millikelvin). But experimenters failed to spot pairing, and many wrote off the prediction as "a theorist's pipe dream," says Douglas Osheroff of Stanford University in Palo Alto, California. Still, in 1972, as a graduate student at Cornell University, Osheroff spotted superfluid helium-3 at 2 millikelvin while trying to study magnetism in solid helium.

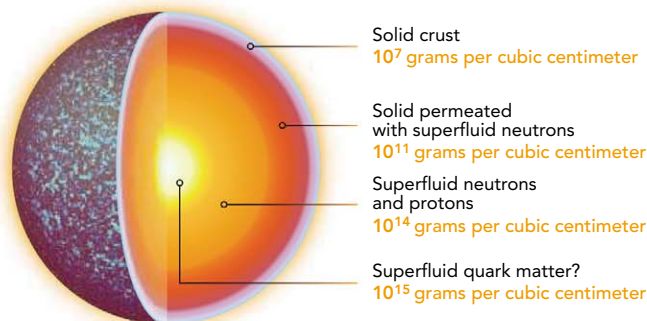
As predicted, pairing in helium-3 proved more complex than in an ordinary superconductor. In the superconductor, electrons with opposite spins join in the simplest way. In helium-3, paired atoms dance around each other and can spin in the same or opposite directions, producing three distinct superfluid "phases." "Helium-3 allowed us to extend our understanding of the implications of the BCS theory," says Osheroff, who shared the 1996 Nobel Prize with his advisers, Robert Richardson and David Lee.

Now, physicists are pushing the bounds of the BCS theory even further in experiments with ultracold gases of atoms. Since the 1990s, scientists have used laser light, radio waves, and magnetic fields to trap and cool atoms to billionths of a kelvin. In 2005, Deborah Jin and colleagues at JILA, a laboratory run jointly by the University of Colorado, Boulder, and the National Institute of Standards and Technology, used the technique to achieve a BCS state of paired atoms of potassium-40. Other groups quickly followed suit.

"Cold fermionic gases are ideal implementations of the ideas of BCS," says Wolfgang Ketterle, an experimenter at MIT. And they let researchers test the theory in new ways. For

example, they have resolved a debate about the relationship between the BCS state and another form of superfluidity. In 1938, physicists discovered that, when liquefied, the heavier isotope of helium, helium-4, formed a free-flowing superfluid below 2.2 kelvin. Helium-4 atoms have no spin and, like all particles with 0, 1, 2 ... units of spin, are known as bosons. Unlike fermions, bosons prefer to crowd into a single quantum wave. So superfluidity in helium-4 stems from a phenomenon called Bose-Einstein condensation, in which particles cram into one quantum wave with no need for pairing. In 1995, physicists achieved Bose-Einstein condensation in cold-atom experiments, too.

Unearthly Applications



Chock-full. Superconductivity theory predicts that a neutron star may contain free-flowing superfluids of paired neutrons and paired quarks.

Are the BCS mechanism and Bose-Einstein condensation related? Bardeen argued they weren't; others reasoned they were. Now, cold-atom experiments have settled the issue, as experimenters can tune a gas of fermions between a BCS state of overlapping pairs and a Bose-Einstein condensate of tightly bound bosonic molecules. "It's not black and white; Bose-Einstein condensation and BCS are two ends of a spectrum," says Ketterle, who shared the 2001 Nobel Prize for achieving Bose-Einstein condensation in cold atoms.

The BCS theory has even seeded breakthroughs in particle physics. It underscored the importance of spontaneous symmetry breaking, which occurs when the basic interactions in a system possess a symmetry that's lost when the system settles into its lowest-energy state. For example, the forces between atoms in a magnet do not favor any one direction, yet all the atoms spontaneously align to minimize their energy. In a superconductor, the mere emergence of a quantum wave of electron pairs breaks a more abstract symmetry called "gauge invariance."

Particle physicists have employed this idea to surprising ends. In 1961, Yoichiro Nambu of the University of Chicago used it to explain the masses of and interactions between hefty protons and neutrons and light particles called pions. Following the BCS theory closely, Nambu imagined that space is filled with a quantum wave of proton-antiproton pairs and neutron-antineutron pairs. The wave, or condensate, then pulls on a proton or neutron to give it mass, whereas the particles in the condensate form the pions. Such work won Nambu a share of the 2008 Nobel Prize.

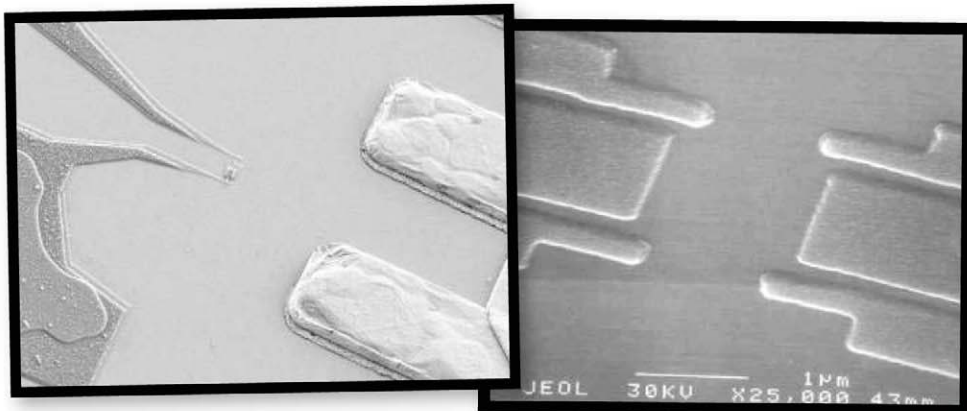
In 1967, Steven Weinberg similarly argued that the electromagnetic force and the so-called weak nuclear force are two aspects of one thing. That's not obvious, as the electromagnetic force extends infinitely far and the weak force reaches only across the nucleus. Weinberg imagined a condensate that drags on the particles that convey the weak force, which he dubbed the W and Z bosons, to make them massive and short-ranged, whereas the photon, which conveys the electromagnetic force, remains massless. "Nambu was inspired by the ideas of superconductivity; I was inspired by Nambu," says Weinberg, now at the University of Texas, Austin, who shared the Nobel Prize in 1979.

That theory proved on the mark in 1983 when the W and Z bosons were discovered with the predicted masses. And the simplest form of Weinberg's condensate would be a quantum field made up of the famed Higgs boson—the most sought-after prize in particle physics. The notion of symmetry breaking has become so essential to particle physics that "all attempts to go beyond the standard model play with symmetries, some of which are broken and some of which are not," Weinberg says.

Superconductivity didn't entirely alter the views of one prominent physicist. Kamerlingh Onnes, who died in 1926 at age 72, never quite accepted that the resistance of a superconductor went completely to zero or fully embraced quantum mechanics, says Van Delft. "He was more or less frozen in the classical world and unable to go down that path," Van Delft says. Still, after a century, one can only marvel at how far Kamerlingh Onnes's discovery has led others.

—ADRIAN CHO

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NEWS

Search for Majorana Fermions Nearing Success at Last?

Researchers think they are on the verge of discovering weird new particles that borrow a trick from superconductors and could give a big boost to quantum computers

IT HAPPENS OVER AND OVER AGAIN in particle physics: Theorists predict the existence of a particle and then, sometime later, experimenters find it. Neutrons, positrons, neutrinos, pions, W and Z bosons, and other subatomic denizens all existed on paper before researchers spotted them, and right now physicists at particle accelerators at Fermilab in Illinois and CERN near Geneva, Switzerland, are hoping that the long-sought Higgs boson will follow suit.

But this well-trodden path is virtually unknown among those scientists who study condensed matter, the stuff of our everyday world. In most materials, defects, impurities, and other imperfections generate too much noise for researchers to spot the vanishingly small signals of ephemeral particles. Even so, one of the most fleeting of all may be on the point of discovery, more than 70 years after it was first proposed.

In recent years, theoretical physicists have suggested that a handful of exotic materials could give rise to this never-before-seen type of particle, known as a Majorana fermion. Now experimental groups around the world are racing to spot it, using devices made in most cases with superconducting materials. And it looks as if some groups are closing in fast or may even have bagged Majoranas already.

“My bet is that if there is something to find, we’ll see it within the next 5 years,” says David Goldhaber-Gordon, an experimental physicist at Stanford University in Palo

Alto, California. Adds Michael Freedman, a mathematician turned theoretical physicist at Station Q, a collaborative research center between Microsoft and the University of California (UC), Santa Barbara: “This is the decade for Majorana fermions. I am extremely optimistic they will be found.”

If they exist, the novel particles are expected to display fundamentally new properties that could open a new window into the mysterious world of quantum mechanics. Their behavior is also expected to make Majorana fermions ideally suited to be stable bits of information in a quantum computer, something that has eluded researchers for decades. “It’s the most exciting thing that has happened in fundamental physics in a long time,” says Leo Kouwenhoven, an experimental physicist at Delft University of Technology in the Netherlands.

Mind-bending math

The notion of Majorana fermions arose as quantum mechanics took shape in the early 20th century as a way to explain the seemingly contradictory behavior of elementary particles, which behaved both as particles and as waves. Researchers had shown that elementary particles have intrinsic properties called charge and spin. Charge is just the familiar electrical property that makes electrons negative, protons positive, and atoms neutral. Spin is a type of rotational momentum related to the magnetic behavior of charged particles. Physicists at the time also knew that elemen-

Majorana detectors? Those in use include tiny transistors (*far left*) and quantum interferometers.

tary particles come in two families: bosons, such as photons, and fermions, such as electrons, that have different groupings of spin.

In 1926, Austrian physicist Erwin Schrödinger came up with an equation that describes how quantum matter changes over time. Two years later, a young English physicist named Paul Dirac tweaked Schrödinger’s equation to make it apply to fermions, such as electrons, that move at speeds near that of light. The expansion integrated quantum mechanics for the first time with Einstein’s special theory of relativity.

Dirac’s new equations also implied the existence of antimatter, matching each fundamental particle with an antiparticle that would annihilate it if the two should ever meet. To their surprise, physicists realized that certain particles, including some photons, could serve as their own antiparticles and annihilate themselves. But fermions weren’t thought to be among them.

Then the story took a twist. In some cases, Dirac’s equations produced results involving imaginary numbers, which some physicists considered inelegant. That’s where a young, gifted Italian physicist named Ettore Majorana entered the picture. In 1937, Majorana modified Dirac’s equations in a way that banished imaginary numbers but had its own mind-bending implications. Most importantly, it allowed for the existence of an entirely new class of fermions—the Majorana fermions—that, unlike traditional fermions, would be their own antiparticles.

Closing in

Majorana never knew what became of his idea. A year after publishing his paper reworking Dirac’s equation, he disappeared mysteriously and was never heard from again. His own favorite candidate for possible Majorana particles, neutrinos, remains a tantalizing possibility today. Massive detectors, such as one deep in the Apennine Mountains of the Abruzzo region in Italy, have been running for years in the hope of spotting a so-called neutrino double-beta decay, which could clinch neutrinos’ Majorana status.

Now condensed-matter physicists are getting in on the hunt and relishing the chance to discover new particles. “That would be pretty cool if we could out-CERN CERN,” says Charles Marcus, a condensed-matter physicist at Harvard University.

It won’t be easy. Majorana fermions

Superconductivity

can't exist just anywhere. To harbor them in all but a few esoteric cases, the material must allow the particles to behave as if they had no spin. It must also force electrons in the materials to pair up as they do in superconductors, in which the pairing allows the electrons to surf through the material without electrical resistance. But whereas superconductors can "glue" electrons together in several different ways, Majoranas can use only one type: an attraction known as chiral p-wave symmetry, which causes electrons with the same spin to pair up. Finally, the electron pairs will hold together only if the material exists as either two-dimensional sheets or one-dimensional wires.

All known fermions have spin, and only a handful of materials meet the other criteria. So for decades the prospects for finding Majorana fermions in materials looked slim at best. "It was a very hard sounding problem," says Jason Alicea, a theoretical physicist at UC Irvine.

Help came from physicists who study how electrons behave in groups. Usually, electrons are like pedestrians on a New York City street, traveling every which way. But like a flash mob that breaks out into a coordinated song and dance routine, groups of electrons can correlate their behavior due to simple interactions between the spins and charges of neighbors. Those interactions can give rise to emergent particles—also called quasiparticles—with characteristics very different from the simple, well-known properties of single electrons, notes Amir Yacoby, an experimental physicist at Harvard. "When you put many together, the collective behavior is fundamentally different," he says.

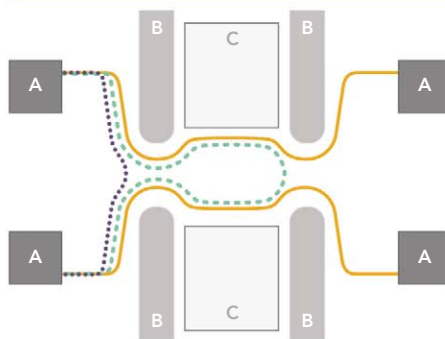
In some semiconductors, for example, electrons moving through strong magnetic fields can jointly create quasiparticles with a charge less than that of an electron—a phenomenon physicists discovered in 1982 and called the fractional quantum Hall effect (FQHE). Initially, those fractional charges all had odd-numbered denominators, such as $1/3$ or $3/7$. In 1987, however, researchers led by Robert Willett at Bell Laboratories in Murray Hill, New Jersey, discovered an FQHE quasiparticle with a $5/2$ charge in a semiconductor crystal of gallium arsenide. Four years later, a pair of theorists at Yale University suggested that this $5/2$ quasiparticle could be a Majorana fermion.

Condensed-matter physicists sensed a discovery in the wings. And now a handful of groups appear to be close to nailing it down. Two years ago Willett, a physicist working

with crystal growth experts Loren Pfeiffer and Ken West of Princeton University, reported measuring signals like those expected from Majorana fermions in the $5/2$ FQHE state. They had built a tiny device known as an interferometer on a gallium arsenide chip tailored to generate the $5/2$ state. Interferometers measure the interference pattern of electromagnetic waves, often visualized as a set of light and dark stripes produced by interfering beams of photons. Willett's team looked at the interference patterns produced by emergent particles in the $5/2$ state.

Theory suggested that Majoranas should appear to have a charge $1/4$ that of an electron ($e/4$): the value researchers see in electrical readings from chiral p-type superconductors. Also in theory, this $e/4$ signal should oscillate and should be interrupted by patches without any electrical activity at all. In a pair of papers

Majorana Interferometer



Quantum detour. Electrodes (A) and sides (B) steer particles around a semiconductor chip. Occasionally, particles hop from the most common path (yellow) into others (blue, purple) or even a superposition of both paths, creating a telltale interference pattern.

published in 2009 and 2010, Willett and his colleagues reported that they had seen the oscillating $e/4$ signal. But in places where the bare patches should be, they saw instead an oscillating charge of $e/2$.

Freedman calls Willett's work "beautiful physics." But he and others say the data remain noisy. "Bob is shaking the right tree," Marcus says. "Something fell out of the tree. But is it an apple?" More results are needed, Marcus says.

Yacoby agrees. He, too, has teamed up with Pfeiffer and West and also appears to be closing in on Majoranas in the $5/2$ state. Using a layered gallium arsenide chip but a different monitoring method, the group reported in *Nature* earlier this year that they had also spotted an $e/4$ signature. That's promising,

but more evidence is needed, Yacoby says: $e/4$ charge alone is "necessary but not sufficient" to prove the existence of the $5/2$ state and the presence of Majorana fermions.

Hot pursuit

The search for Majorana particles is heating up in other solid-state systems as well. In 2008, theorists Liang Fu and Charles Kane of the University of Pennsylvania argued that depositing a widely available type of superconductor known as an s-wave superconductor atop a novel material known as a topological insulator would be a simpler way to make them appear.

Topological insulators conduct electricity only on their surfaces but act as insulators throughout their bulk; shaped into two-dimensional sheets, they conduct only at their edges. Fu and Kane's calculations suggested that the interactions between the two materials would force quasiparticles to form with p-wave glue and behave as if they had no spin—the exact recipe needed to make Majoranas. "That idea jump-started the field," says Marcel Franz, a theoretical physicist at the University of British Columbia, Vancouver, in Canada.

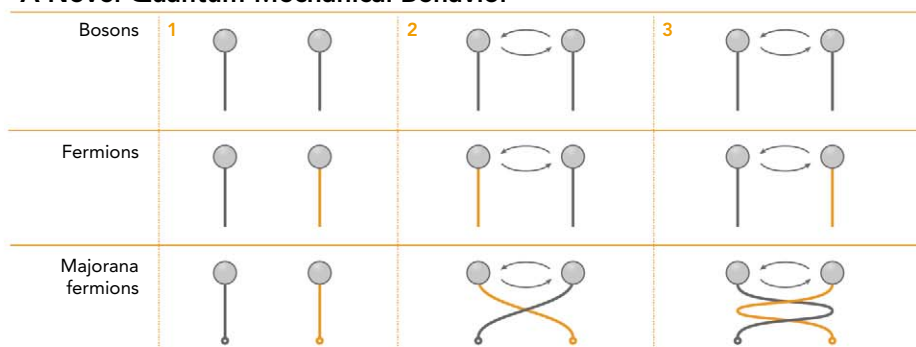
Sankar Das Sarma, a theorist at the University of Maryland, College Park, and others ran with Fu and Kane's ideas. They soon discovered that topological insulators aren't necessary; a particular type of semiconducting nanowire placed atop an s-wave superconductor would work just as well. The key feature needed in the nanowires was a property known as high spin-orbit coupling, in which an electron's orbital motion as it circles an atomic nucleus is strongly linked to its spin.

As fortune would have it, Kouwenhoven's lab at Delft had experimented with exactly that setup. The group members were experts at making pristine nanowires from a semiconductor alloy called indium arsenide (InAs), whose ability to create near-perfect electrical contacts with certain metals makes it an excellent test bed for studying how electrical charges move between different materials.

In 2005, Kouwenhoven's postdoc Silvano De Franceschi had placed an InAs nanowire on top of a superconductor and measured how currents flowed through the two materials. The results fell in line with what was expected, so the researchers published their results and moved on. "We thought that was the end of the work," Kouwenhoven says.

Das Sarma's paper, along with another by a team led by Yuval Oreg of the Weizmann Institute in Rehovot, Israel, made

A Novel Quantum-Mechanical Behavior



Keeping track. When two bosons trade places, there is no change in their quantum mechanical state. Normal fermions change the sign of their mathematical “wavefunction” from positive to negative (red) with each switch, returning to their original state after two switches. Majorana fermions “remember” their path.

them look again. Because InAs has strong spin-orbit coupling, Kouwenhoven realized, the new work suggested that a setup like De Franceschi’s ought to be a fruitful hunting ground for Majorana fermions. “It put us at the center of attention,” Kouwenhoven says.

Kouwenhoven’s team raced to recreate its earlier nanowire experiments and carefully added a strong magnetic field. Too high a field would destroy a material’s ability to superconduct. But one just below that level, according to Fu and Kane’s theory, should produce Majorana fermions at the ends of the wires.

If the exotic quasiparticles are there, theory suggests physicists should be able to detect them by measuring (with, say, the metal tip of a scanning tunneling microscope) how readily they conduct electrons. Normal particles have a small electrical resistance, whereas Majoranas should have none. Physicists should also see unique electronic signatures when Majorana fermions are created in the heart of so-called superconducting quantum interference devices.

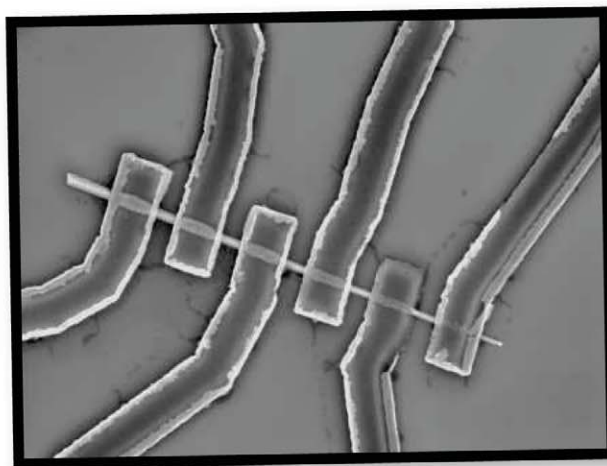
Kouwenhoven says he and his group are looking for such signatures. They haven’t spotted them yet in InAs and are now testing nanowires with higher spin-orbit coupling, eager for the right mix of properties. “We hope to hit a sweet spot,” Kouwenhoven says.

A Majorana computer?

They aren’t alone. Alicea, Franz, and others who are plugged into the field say proposals from other academic groups are pouring in to spot Majorana fermions in nanowire setups and a handful of other promising solid-

state systems. If any of the techniques succeed, researchers will gain the ability to play with particles with quantum behavior unlike anything else ever seen.

Most notably, Majorana fermions are expected to break new ground in an area called quantum-mechanical exchange statistics. The phrase describes how the quantum state of two identical particles changes as the particles change places. All known bosons



Wired for discovery. Superconducting electrodes atop an indium arsenide wire may create Majorana fermions at each end.

and fermions behave in a way that, in effect, erases the history of the motions; Majorana particles, by contrast, will leave tracks.

To visualize how this works for bosons, Marcus says, imagine holding two pegs, each with a string dangling from it (see figure, top of page). Move the two pegs around each other to trade places and the strings follow. Swap them again and the pegs and strings end up back where they started, leaving no clue as to their earlier path. Things are slightly more complex with standard fermi-

ons, but after two exchanges they return to their original state as well.

Not so with Majorana fermions. In their case, it’s as if each string starts out taped to the floor below its peg. If you move the two pegs past each other in the same direction, the strings will wind around each other—and anyone who examines them can tell whether the pegs went clockwise or counterclockwise. Similarly, when Majoranas move, they change their quantum state in a manner that reflects the path they’ve taken. The result, researchers say, could be an entirely new way of studying the subtleties of quantum mechanics. “This is a really big fish out there waiting for us to find it,” Alicea says.

It may have practical applications, too. Several years ago, Freedman and his colleagues reasoned that such unique exchange behavior could be used to encode information. In that case, Majorana fermions could serve as quantum bits of data, or qubits, in a quantum computer. Qubits are quantum analogs to the binary 0s and 1s in your standard desktop, laptop, or cell phone. Unlike conventional bits, qubits store information not as either a 0 or a 1, but as a superposition of both 0 and 1. As a result, a quantum computer containing only a few hundred qubits can carry out certain kinds of calculations faster than the most powerful supercomputers.

Real-world qubits, however, are extremely delicate. The slightest hint of heat or other perturbation can destroy the superposition, rendering them useless. That fragility has made complex quantum computers difficult to create. The braided Majorana particles, by contrast, are expected to shrug off such environmental wiggles. “In theory, they should be more robust qubits,” Franz says.

Freedman, Alicea, and others have designed systems of nanowires and other devices to manipulate Majorana fermions and encode information. But few physicists expect to see working models anytime soon. That’s just fine with Marcus, who says he looks forward to the challenge. The hunt for Majorana fermions “is not a Holy Grail problem, where once you get it there’s nothing left to do,” he says. Researchers who discover them will still face the arduous task of learning to control them well enough to build a quantum computer. “I’ll see you in 20 years when we get all this to work,” Marcus says.

—ROBERT F. SERVICE

The Challenge of Unconventional Superconductivity

Michael R. Norman

During the past few decades, several new classes of superconductors have been discovered that do not appear to be related to traditional superconductors. The source of the superconductivity of these materials is likely different from the electron-ion interactions that are at the heart of conventional superconductivity. Developing a rigorous theory for any of these classes of materials has proven to be a difficult challenge and will remain one of the major problems in physics in the decades to come.

Superconductivity is an exotic state of matter that has intrigued scientists ever since its discovery in mercury in 1911 (1). It is sobering to realize that after 100 years, there are whole classes of superconducting materials that we still do not fully understand. Even for the first (“conventional”) superconductors, it took half a century to develop a theory, starting with the groundbreaking work of Cooper in 1956 (2) and Bardeen, Cooper, and Schrieffer (BCS) in 1957 (3). These ideas rapidly developed into a rigorous theory several years later (4) because for electron-ion interactions, a controlled many-body perturbation expansion is possible that greatly reduced the complexity of the problem (5). Unfortunately, no such luxury exists for the more recently discovered unconventional superconductors, and therefore the development of rigorous theories explaining these materials has proven to be a real challenge.

Conventional and Unconventional Superconductors

To appreciate these issues, we need to first understand what superconductors are all about, and how unconventional ones differ from their more conventional counterparts. Superconductors are not only perfect conductors (their electrical resistance drops precipitously to zero below a transition temperature T_c), but also exhibit the so-called Meissner effect (6), where they expel magnetic fields. As noted by Fritz London (7), this implies that electrons in superconductors behave in a collective manner. Bosons, which have integer values of a fundamental property known as spin, can behave in this fashion, whereas electrons, which are fermions that have half-integer spins, typically do not. In 1956, this apparent contradiction was resolved by Leon Cooper (2), who demonstrated that the presence of even an arbitrarily small attractive interaction between the electrons in a solid causes the electrons to form pairs. Because these “Cooper pairs” behave as effective bosons, they can form something analogous to a Bose-Einstein condensate. Rather than being real-space mole-

cules, however, Cooper pairs consist of electrons in time-reversed momentum states and consequently have zero center-of-mass momentum. Because a pair of identical fermions is antisymmetric with respect to the exchange of one fermion with another, the spin and spatial components of the Cooper pair wave function must have opposite exchange symmetries. Thus, these pair states are either spin singlets with an even-parity spatial component, or spin triplets with odd parity.

The spin singlet pair state with an isotropic spatial component (s-wave) turns out to be the one realized in conventional superconductors (3). Even though electrons repel each other because of the Coulomb force, at low energies there can be an effective attraction resulting from the electron-ion interaction. This can be understood by considering that a metal is formed by mobile electrons detaching themselves from the atoms that form the crystalline lattice (these atoms then become positive ions). Such a mobile electron attracts the surrounding ions because of their opposite charge. When this electron moves, a positive ionic distortion is left in its wake. This attracts a second electron, leading to a net attraction between the electrons. This mechanism

works because the ion dynamics is slow relative to the electrons—a consequence of the fact that the ions are much heavier than the electrons. However, the interaction at shorter times becomes repulsive because of the Coulomb interaction between the electrons; this retardation is what is responsible for limiting T_c (8). Until the discovery of cuprates, the highest known T_c was only 23 K.

The resulting wave function for the pairs turns out to be peaked at zero separation of the electrons—that is, an s-wave state. By an “unconventional” superconductor, we mean one that is not s-wave. Typically, this means that the pair wave function, also known as the order parameter, is not uniform in momentum space, although there can be exceptions, as may be the case for the new iron-based superconductors.

^3He —The First Unconventional Superfluid

The first unconventional material was not a superconductor, however, but rather superfluid ^3He (9). Because the atoms are neutral objects in this case, there is no analog of the electron-ion interaction mentioned above. In fact, the helium atoms behave as hard-core objects, which acts to suppress s-wave pairing. One of the first proposals to explain the pairing was based on van der Waals attraction between the atoms at larger separations (10), resulting in the formation of d-wave pairs. This pair state suppresses the influence of the hard core because it has a node for zero separation of the atoms, and the d-wave state optimizes the separation of the atoms to take advantage of the van der Waals attraction. A p-wave state, which has a node as well, was also proposed, but because of fermion antisymmetry, this odd-parity state is associated with spin triplet pairs. In this case, the attraction was speculated to be a result of exchange forces (11); ^3He was thought to be nearly ferromagnetic, so an atom would prefer to align its spin with its pair partner. When superfluidity was seen in ^3He several years later,

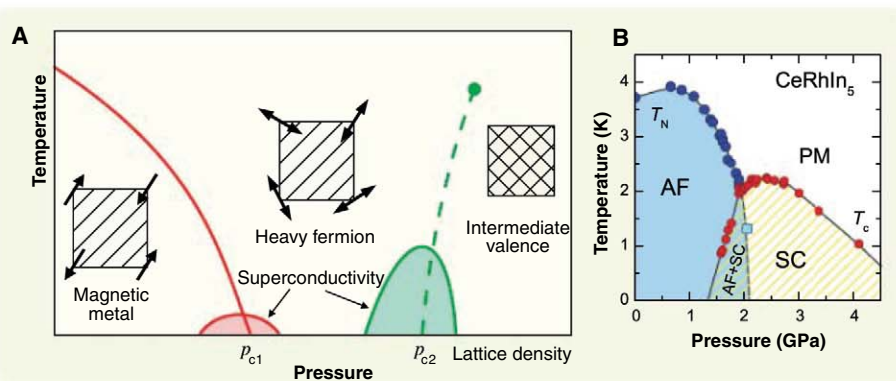


Fig. 1. (A) Schematic phase diagram of the heavy-fermion material $\text{CeCu}_2\text{Si}_{2-x}\text{Ge}_x$. Two superconducting domes are present, one (red) associated with a quantum critical point of an antiferromagnet (at p_{c1}), the other (green) with a volume collapse transition (at p_{c2}) (58). **(B)** Phase diagram of CeRhIn_5 (temperature versus pressure). The superconducting (SC) phase with a critical temperature T_c abuts an antiferromagnetic (AF) phase with a Néel temperature T_N , with a small coexistence region in between and a paramagnetic (PM) phase at higher temperatures (59).

it was soon realized that this was indeed a consequence of p-wave pairing (12). Two different superfluid phases, known as A and B, exist below a few millikelvin; these were also explained on the basis of exchange forces (13). Given the simplicity of the liquid state, the pair interaction was eventually quantified using known normal-state interaction (Landau) parameters. This analysis revealed that many factors contribute to the pair interaction, including density, spin, and transverse current interactions (12). Hence, it can be misleading to claim that one mechanism is the sole cause of pairing in unconventional superconductors.

Heavy-Fermion Superconductors

Of course, neutral atoms are not the same as charged electrons. Surprisingly, superconductivity with $T_c = 0.5$ K was discovered in 1979 in CeCu_2Si_2 (Fig. 1A) by Steglich's group (14), and then in several uranium alloys such as UPt_3 and UBe_{13} (15) a few years later. These materials contain magnetic 4f and 5f ions, which previous experience indicated would have been incompatible with superconductivity. After all, magnetic impurities are well-known sources of pair breaking in conventional superconductors (16). But these materials are more intriguing because the ions exhibit the Kondo effect. In such systems, the mobile conduction electrons have a tendency to form a bound resonance with the localized f electrons of the magnetic ions (17). When these magnetic ions form a regular lattice, this "Kondo" lattice is characterized by an electronic specific heat coefficient of order 1000 times that of conventional metals such as copper, with a correspondingly large spin susceptibility. This "heavy" Fermi liquid can form a number of ordered states, including magnetic order and, intriguingly, unconventional superconductivity (15).

Given some similarities with ^3He , where the liquid phase is near a solid phase with magnetic order, these heavy-fermion metals were initially thought to be p-wave superconductors as well (18). But subsequent work has indicated that this is a complex problem (19). First, the concept of s-wave, p-wave, etc., must be taken with a grain of salt because of the presence of a crystalline lattice that breaks translational symmetry. Second, not only are multiple f orbitals involved, there are multiple conduction electron orbitals as well. Finally, spin-orbit effects are large for Ce and U ions, and they play a qualitatively different role than in light ^3He atoms. In fact, although more than 30 years have elapsed since the discovery of heavy-fermion superconductors, the actual symmetry of the Cooper pairs of any of them has not yet been unambiguously determined.

Perhaps the closest we have come is UPt_3 (20). This material, like ^3He , has several superconducting phases (Fig. 2A). Because of the hexagonal symmetry of the lattice and the fact that the spin and orbital angular momentum of the pairs are linked because of spin-orbit cou-

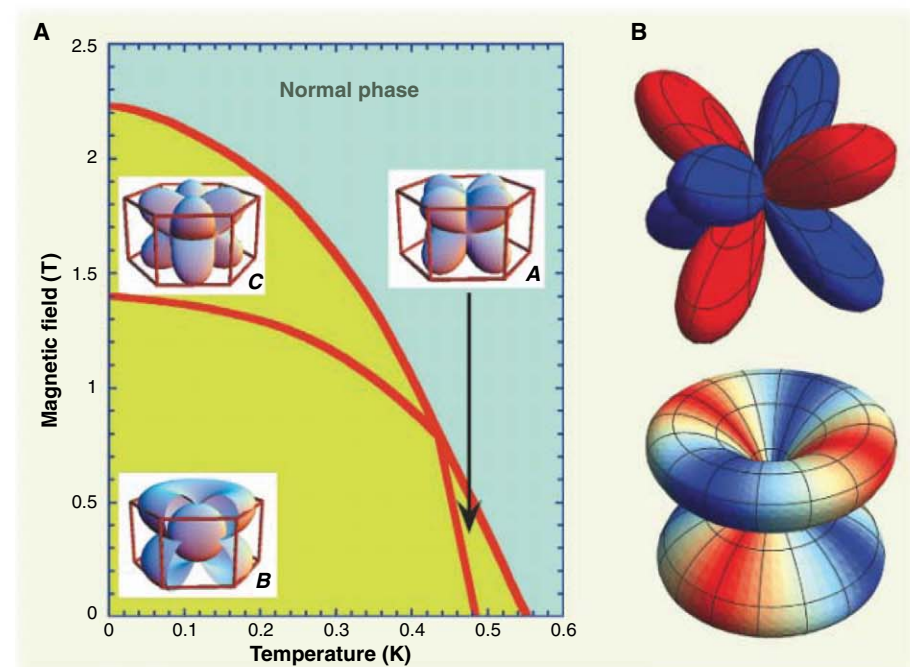


Fig. 2. (A) Phase diagram of UPt_3 . Three different superconducting phases, A, B, and C, are present (60). **(B)** f-wave (E_{2u}) Cooper pair wave function in three-dimensional momentum space proposed for phase A (top) and phase B (bottom) on the basis of phase-sensitive tunneling (61). In comparison, an s-wave wave function characteristic of conventional superconductors would be a simple sphere.

pling, this implies that either two different superconducting states have nearly identical transition temperatures, or that the order parameter is doubly degenerate. The latter is more likely, as it is thought that the small temperature range separating the two phases at zero magnetic field is a result of a weak lifting of the hexagonal symmetry of the lattice caused by the presence of small magnetic moments on the uranium ions. A variety of thermodynamic data indicate that the order parameter probably vanishes along lines that are perpendicular to the c axis of the crystal. This restricts the order parameter symmetry to be either E_{1g} ("d-wave") or E_{2u} ("f-wave"). Very recently, measurements have been carried out that are sensitive to the phase of the order parameter (21). They indicate that the order parameter behaves as $\exp(2i\phi)$, where ϕ is the azimuthal angle within the hexagonal plane. If a line of nodes is indeed present, this rules out E_{1g} in favor of E_{2u} . Such an f-wave order parameter is indeed an exotic beast (Fig. 2B). But it is highly doubtful that the actual order parameter is so simple: UPt_3 has a complex Fermi surface (which separates occupied from unoccupied states) formed from five different energy bands, and the spin-orbit coupling is so strong that even the single-particle states are best characterized by states of total angular momentum J .

Less is known about other heavy-fermion superconductors. Much recent work has gone into the so-called 115 series, with a formula unit CeXIn_5 where X is a transition metal ion (Fig.

1B). Available data are consistent with a non-degenerate order parameter that is singlet in nature, leading to the speculation of d-wave pairs. But to date, no phase-sensitive measurements have been performed. Of perhaps greater interest are the plutonium analogs, one of which has a T_c of 18 K, almost an order of magnitude higher than those previously known for heavy-fermion superconductors (22). Even less is known about this material given the challenges of working with plutonium, but its discovery indicates that perhaps even more striking examples await us in the future. Already there are heavy-fermion superconductors, such as UGe_2 and URhGe , that are simultaneously ferromagnetic and superconducting. And perhaps related to these systems is Sr_2RuO_4 (23), a multiband layered transition metal oxide that appears to be a p-wave superconductor, although the exact nature of the order parameter is still being debated. Available phase-sensitive measurements are certainly consistent with a p-wave state (24).

A fundamental difference from ^3He , though, is that most heavy-fermion superconductors are actually nearly antiferromagnetic, and in some cases (most notably in the Ce 115 series), superconductivity and antiferromagnetism coexist (Fig. 1). This was realized in 1985 when strong antiferromagnetic spin correlations were seen by neutron scattering in UPt_3 (25), leading to the publication of three theoretical papers advocating that antiferromagnetic spin fluctuations were the source of d-wave superconductivity (26–28).

Superconductivity

Superconductivity seems to be maximal at a point where magnetism disappears (Fig. 1); this was first appreciated in 1998 when superconductivity was discovered under pressure in CePd_2Si_2 and CeIn_3 (29). A phase transition suppressed to zero temperature is known as a quantum phase transition (30), and it is thought that critical fluctuations associated with this quantum critical point could be the source of pairing (29).

Cuprates

In the same year, 1986, that the above-mentioned spin fluctuation papers were published, a small group from an IBM lab in Zürich made a startling discovery: superconductivity near 40 K in the layered cuprate $\text{La}_{2-x}\text{Ba}_x\text{CuO}_4$ (31). At first, this result did not attract much attention [in the past, there had been a number of claims of “unidentified superconducting objects” (USOs)]. But after its reproduction by several groups, a flurry of activity was unleashed, leading shortly to the discovery of superconductivity above 90 K in $\text{YBa}_2\text{Cu}_3\text{O}_7$ (32). The technological implications were profound, given that liquid nitrogen, instead of helium, could be used for cooling. Surpris-

face of physics (34). Philip Anderson, the Nobel laureate, proposed instead that cuprates would exhibit a novel phase of matter where the spins formed a liquid of singlets—the so-called RVB (resonating valence bond) state—on the basis of previous work he had done in the 1970s on frustrated magnets. The name RVB was motivated by the classic work of Linus Pauling on benzene rings, where the carbon bonds fluctuate between single and double bonds. Anderson argued that such an RVB state was the consequence of several unique properties of cuprates: The materials are quasi-two-dimensional, the copper ions have spin $\frac{1}{2}$, and the parent phase is a Mott insulator—that is, a state with an odd number of electrons per unit cell that is insulating because of many-body correlations. These effects, he speculated, would act to melt the expected antiferromagnetic (Néel) lattice into this spin-liquid phase. Upon carrier doping, these singlets would become charged, resulting in a superconducting state. Although the original proposal was for a uniform RVB (s-wave) state, subsequent work found that the free energy was actually minimized for a d-wave state (35). Although undoped cuprates

is the more appropriate. The lack of resolution of this debate is connected to the fact that for electronic-only models, we do not have a controlled perturbation expansion to work with, as we do for the electron-ion interactions underlying conventional superconductors.

Moreover, cuprates are complex systems with a variety of important interactions, including electron-ion. This has become increasingly obvious in attempts to explain their phase diagram (Fig. 3A). After the Néel order is destroyed by doping, there are four apparent regions of the phase diagram: (i) a pseudogap phase where an energy gap is present, (ii) a strange metal phase characterized by a resistivity linear in temperature, (iii) a Fermi liquid phase with largely normal transport properties, and (iv) a d-wave superconducting phase. In the RVB approach, the pseudogap is a spin gap phase resulting from spin singlet formation, whereas in the spin fluctuation approach, it is a fluctuating version of the Néel phase. But experiments now indicate an intriguing variety of phenomena associated with the pseudogap phase, including nematic (39) correlations (where the C_4 rotational symmetry of the square lattice is spontaneously broken), and a novel form of magnetism, arising from either orbital currents or antiferromagnetism that is associated with the oxygen sites in the CuO_2 unit cell of the cuprates (40). At lower temperatures, charge-density wave, spin-density wave, and superconducting correlations become apparent in a variety of measurements. From this very complicated soup, high-temperature superconductivity arises. Because superconductivity is created from the normal state, these phenomena must be understood before we will ever have a true understanding of the origin of high-temperature cuprate superconductivity.

Several authors have pointed out the similarities of the phase diagram of cuprates (Fig. 3A) with that of heavy fermions (Fig. 1). Accordingly, it has been proposed that superconductivity is mediated by quantum critical fluctuations, but again, the nature of the purported quantum critical point (which in the case of the cuprates is “hidden” under the superconducting dome) is being actively debated. Is it associated with antiferromagnetism, charge-density waves, spin-density waves, nematic correlations, orbital currents, or a combination thereof?

Organic Superconductors

If the physics of doped Mott insulators is indeed the key to cuprates (37), this will also have relevance to organic superconductors (41). These materials were discovered well before the cuprates and were an equal surprise to the community; as Bernd Matthias once quipped, “there aren’t any!” Well, they are indeed real, and the two-dimensional variety has a phase diagram intriguingly similar to their cuprate counterpart (Fig. 4A). In the BEDT-TTF salts, one has a lattice of molecular dimers, with one spin $\frac{1}{2}$ degree of freedom per

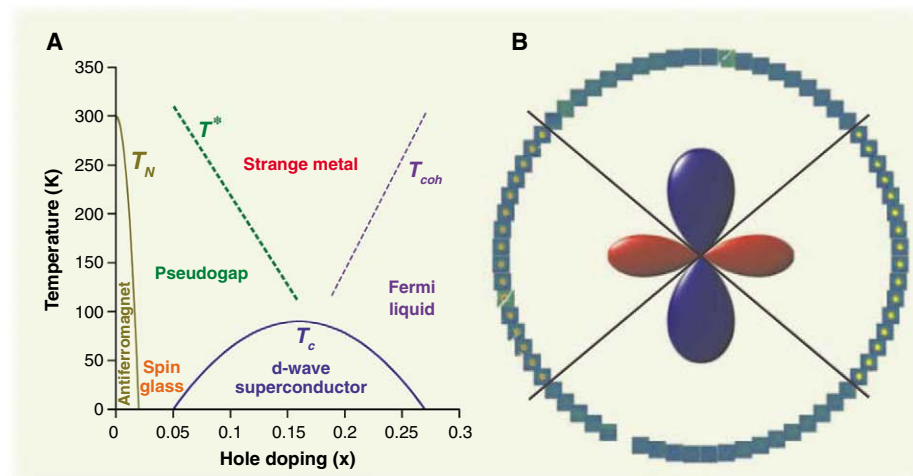


Fig. 3. (A) Schematic phase diagram of the cuprates (62). **(B)** Cooper pair wave function for $\text{YBa}_2\text{Cu}_3\text{O}_7$ as a function of the planar azimuthal angle measured by phase sensitive tunneling (63). The red and blue lobes denote the opposite signs of the $d_{x^2-y^2}$ state. The size difference of the two lobes is due to the orthorhombicity of this material, which leads to mixing in of a small s-wave component.

ingly, this class of materials violated most if not all of the empirical search rules set down by Bernd Matthias; these rules were based on the previous record high T_c materials, which were cubic transition metal alloys, whereas cuprates are obtained by doping carriers into a parent material that is an insulating magnetic oxide. Not surprisingly, theorists speculated that the solution for the cuprate puzzle was a two-dimensional variant of the d-wave superconductivity mentioned above in the heavy-fermion context, $d_{x^2-y^2}$ symmetry (33).

But before this, a very different theory appeared that, for better or worse, would change the

were soon found to form a Néel lattice (but with a reduced moment), a few percent of doped holes was sufficient to destroy this state (Fig. 3A).

What is unquestionable is that the exchange interaction J for cuprates is very large, on the order of 1400 K, and as such is an attractive source for pairing. This became very relevant in the mid-1990s, when it was shown by phase-sensitive tunneling (36) that the pairing state was indeed d-wave (Fig. 3B). But such a large J is also relevant for the more traditional spin fluctuation-based approaches, and there is currently much debate about which of these two approaches, RVB (37) or spin fluctuations (38),

dimer. These are arranged in a triangular fashion, which has a tendency because of frustration to suppress magnetic order. It was indeed such a lattice that was the motivation for the original RVB idea. These materials at ambient pressure typically have a Mott-insulating ground state, becoming superconducting under pressure (42). Available evidence indicates a d-wave state, but as with other unconventional superconductors other than cuprates, the true pairing symmetry has yet to be unambiguously determined. Much of the recent attention regarding these materials has been devoted to those compounds that seem to exhibit a spin-liquid ground state in the Mott phase, as well as to the question of whether a Fermi surface of spin degrees of freedom (a so-called spinon Fermi surface) is indeed realized (43), as originally proposed by Anderson for the cuprates (34). As with the cuprates, there has been an interesting debate regarding RVB versus spin fluctuation approaches for the pairing mechanism.

Iron-Based Superconductors

This brings us to the newly discovered iron-based pnictide and related superconductors (44). Although involving iron rather than copper, and arsenic rather than oxygen, there are enough similarities to cuprates for these debates to again occur for this class of compounds (45). Unlike the cuprates, it appears that all five of the 3d orbitals of the iron are involved in the electronic structure near the Fermi energy. Although the undoped material is also antiferromagnetic, unlike in the cuprates, it is metallic. With doping, the magnetic state is suppressed and a high-temperature superconducting phase appears (Fig. 4B). It has been speculated that the order parameter is a so-called $s_{\pm}$ state, where the Fermi surfaces around the Γ point of the Brillouin zone have an order parameter with one sign, and those around the M point of the zone the opposite sign (46). Like the cuprates, and as has been speculated for the heavy fermions, this order parameter satisfies the condition that it changes sign under translation by the magnetic ordering vector of the parent phase. Such a state tends to remove the detrimental effects of the on-site Coulomb repulsion between the electrons, and naturally appears in spin fluctuation models. The jury, though, is still out on this question. Certainly this order parameter is consistent with what is known from angle-resolved photoemission, which shows rather isotropic energy gaps around each Fermi surface (47), but thermal conductivity (48) and other measurements indicate for certain dopings—gaps that appear to be d-wave-like in nature. And although the correlations appear to be weaker than in the cuprates because of the multi-orbital nature of the electronic structure (the electrons can avoid one another by occupying different d orbitals), there has been a rather heated debate on whether the magnetism is itinerant (as in the spin-density wave state in chromium) or more localized (as in

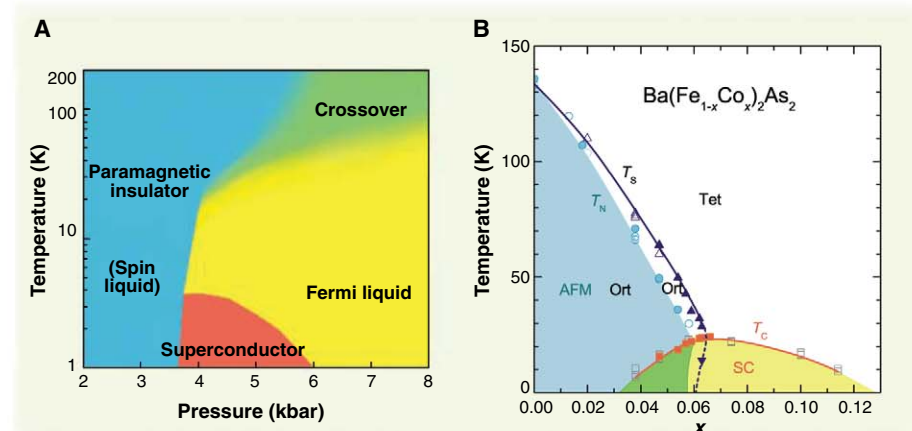


Fig. 4. (A) Phase diagram of the organic superconductor $\kappa\text{-(ET)}_2\text{Cu}_2(\text{CN})_3$ (temperature versus pressure) (64). No magnetic order has been detected in the Mott insulator phase. (B) Phase diagram (temperature versus doping x) of the pnictide superconductor $\text{Ba(Fe}_{1-x}\text{Co}_x)_2\text{As}_2$ (65). The antiferromagnetic (orthorhombic) phase occurs below T_N (T_s) and is labeled by AFM (Ort). The normal (tetragonal) phase is denoted by Tet, and the superconducting phase, which occurs below T_c , by SC. Note the similarities of the phase diagrams presented in Figs. 1, 3, and 4.

the case of the cuprates). Interestingly, strong nematic effects similar to what have been observed in the cuprates have been seen in these materials as well (49).

Theory and Outlook

From a theoretical point of view, it has become increasingly obvious that unconventional superconductivity is a very tough problem. Even for the simple case of one d orbital with an on-site Coulomb repulsion, which has been considered by many to be the minimal description for cuprates, we do not know whether this model is indeed superconducting. Quantum Monte Carlo simulations of this model have given conflicting results, the issue being the infamous fermion sign problem that plagues such simulations. Even if the model turns out to be superconducting, there are many who feel that it is not sufficient—for instance, because of the rather large electron-ion effects that exist even for cuprates (50), or because a three-band model may be necessary to describe the physics (51). Indeed, multiband models are unavoidable in many cases, most prominently for heavy fermions, ruthenates, and pnictides. And, as opposed to conventional superconductors, in electronic-only mechanisms there is no really controlled perturbation theory to work with. Theorists have attempted to get around this by suggesting that an effective expansion parameter might exist, exploiting the fact that the collective spin fluctuations are “slow” relative to individual fermion degrees of freedom. On the other hand, it is well known that some higher-order terms are as large as the lowest-order term in such theories (52). Even in the RVB approach, most of the work has been done at an effective mean-field level reminiscent of BCS theory. Attempts to go beyond this by considering “gauge” fluctuations associated with constraints on a given copper site (such as no

double occupancy) have met with limited success (37). In both the spin fluctuation and RVB “gauge” approaches, large N expansions (where N is the degeneracy of the electronic states) have been attempted, motivated by earlier work in heavy-fermion materials. But even in heavy fermions where the orbital degeneracy of the f electrons is large, N is typically 2 in the low-energy sector because of crystal field splittings, so the relevance of these approaches is controversial. Even the string theorists have gotten into the act, suggesting that the AdS/CFT (anti-de Sitter/conformal field theory) methods they have used to study certain strong coupling gauge theories may be relevant to condensed matter systems such as cuprates (53). Certainly, in the coming years, there will be increasing attention given to constructing rigorous nonperturbative strong coupling theories in the hope that we can “solve” the problem of unconventional superconductivity, at least to most people’s satisfaction.

Since the discovery of cuprates, several new families of superconductors have been discovered. MgB_2 appears to be a conventional superconductor, and its properties were explained rather quickly by the standard strong-coupling approach for electron-ion interactions (54). It is revealing that this simple material was missed by both the experimentalists and theorists for such a long period of time. Alkali-doped buckyballs also have a substantial T_c near 40 K, and although at first sight their superconductivity appears to be conventional, there is increasing evidence that Mott physics may be involved here as well (55). And the discovery of pnictides again took the community by surprise. Because of this, there is no doubt that new classes of superconductors await our discovery. Whether current beliefs will aid us in this quest remains to be seen. The discovery of large values of the exchange constant J in iridium oxides (56) has led a few of us to

propose that these materials might also be high-temperature superconductors. But attempts to dope these materials have so far not led to a superconducting phase. There are also a number of layered nitrides that have a comparatively high T_c near 26 K (57). Perhaps different variants are out there that will take us into the T_c range of cuprates. Regardless, what is apparent is that such discoveries will only occur with a proper investment in materials synthesis, guided by a good intuition of where to look. If this occurs, the future will indeed be bright.

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REVIEW

The Electron-Pairing Mechanism of Iron-Based Superconductors

Fa Wang¹ and Dung-Hai Lee^{2,3*}

The past three years have witnessed the discovery of a series of novel high-temperature superconductors. Trailing behind the cuprates, these iron-based compounds are the second-highest-temperature superconducting material family known to date. Despite the marked differences in the chemical composition, these materials share many properties with the cuprates and offer the hope of finally unveiling the secret of high-temperature superconductivity. The main theme of this review is the electron-pairing mechanism responsible for their superconductivity. We discuss the progress in this young field and point out the open issues.

In April of 1911, Heike Kamerlingh Onnes and co-workers cooled mercury to the boiling temperature of liquid helium (4.2 K) and observed the abrupt vanishing of its electrical resistance (I). This event marks the discovery of superconductivity. In the ensuing 100 years, many

superconductors were discovered. The highest superconducting transition temperature ($T_c = 135$ K at ambient pressure) has been observed in a family of compounds called the “cuprates,” which are said to exhibit high-temperature superconductivity. In early 2008, Kamihara *et al.* discovered a novel $T_c = 26$ K, iron-based superconducting compound (FeSC) (2); to date, the highest T_c in related superconductors has been raised to 55 K (3). Like the cuprates, these new superconductors are obtained from parent compounds belonging to a magnetically ordered class of materials called antiferromagnets (AFs) by

the process of “doping” (charge injection via chemical substitution). However, FeSCs are more tunable because superconductivity can also be achieved by applying pressure to the parent stoichiometric compounds (4, 5). Many feel that these materials have opened new directions for the search of high-temperature superconductivity; their optimism is reflected by referring to the discovery of the Fe-based superconductors as the beginning of the “Iron Age of high-temperature superconductivity.”

Although the subject of Fe-based high- T_c superconductivity is very young, there are already a vast number of research papers published on this topic; almost all experimental techniques used to study the cuprates have been applied to the new high- T_c materials. In this Review, we focus on one major theme: the electron-pairing mechanism that is responsible for superconducting properties.

Structure and Magnetism

The newly discovered FeSCs come in many chemical compositions. Some of the stoichiometric parent compounds include LaFeAsO (2) (abbreviated as 1111 for its 1:1:1:1 ratio of the four elements), BaFe₂As₂ (6) (the 122 compounds), FeTe (7) (the 11 compounds), and LiFeAs (8) (the 111 compounds). In a very recent development, a number of intercalated FeSe compounds

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$A_x\text{Fe}_{2-x}\text{Se}_2$ (where $A = \text{K}, \text{Cs}, \text{Tl}$) were made, raising the T_c from 8 K for FeSe to above 30 K (9). The common chemical building block of these superconductors is FeX, where $X = \text{As}, \text{P}, \text{S}, \text{Se}, \text{or Te}$. Structurally, FeX forms a trilayer consisting of a square array of Fe sandwiched between two checkerboard layers of X (Fig. 1A, inset). These trilayers are separated by “bridging layers” consisting of alkali, alkaline-earth, or rare-earth atoms and oxygen/fluorine. It is widely believed that superconductivity in this family originates from the electrons of the d orbitals of Fe in the FeX trilayers.

Most of the stoichiometric parent compounds exhibit antiferromagnetism at ambient pressure. With the exception of FeTe, the spatial arrangement of the magnetic moments in an FeX trilayer is represented by the red arrows in Fig. 1B (10–12). This magnetic order has been explained from both the itinerant- (13, 14) and the localized-electron (15) point of view. Along the crystalline c axis (see Fig. 1A for the definition of a , b , and c axes), the nearest-neighbor magnetic moments can be either parallel or antiparallel. This magnetic order couples intimately with a structural distortion. For the stoichiometric (122) system [for example, BaFe_2As_2 (11)], the structural and antiferromagnetic transitions occur at the same temperature (the zero doping axis of Fig. 1E) through a first-order phase transition in which the AF order appears discontinuously. In the low-temperature phase, the ab plane Fe-Fe distance elongates in the direction parallel to the magnetic moment and contracts in the direction perpendicular to it (blue arrows in Fig. 1B). For the (1111) system [for instance, LaFeAsO (10) and CeFeAsO (12)], the structural transition occurs at a slightly higher temperature followed by a magnetic transition (Fig. 1, C and D). Thus, there exists a temperature window in which the stoichiometric (1111) compounds are paramagnetic, but the crystal 90° rotation symmetry is broken by the structural distortion. The above phenomenon suggests that, in samples where static magnetism is suppressed but fluctuating magnetism still exists, the electron-lattice coupling will be enhanced (16). This coupling could impede or assist the electron pairing discussed later in this review.

The AF state is a “semimetal” with equal density of negative and positive charge carriers. This is in sharp contrast with the cuprates where the AF compounds are insulating; however, semimetallicity does not necessarily imply that the electron-electron correlations is weak (17).

The fact that antiferromagnetism needs to be suppressed for superconductivity to thrive (Fig. 1, C to E) also applies to the cuprates, as well as many other materials in nature. It is this fact that leads many to the proposition that dynamic rather than static antiferromagnetism (or AF fluctuations) is favorable for high-temperature superconductivity. This point of view is supported by a recent neutron-scattering experiment which found

that, in $\text{BaFe}_{1.85}\text{Co}_{0.15}\text{As}_2$ (a FeSC), the AF fluctuation is as strong as that of $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$ (a cuprate superconductor) (18).

The Pairing Problem

After the discovery of superconductivity in mercury and other metals, many theories were put forward to explain the phenomenon. Eventually, the Bardeen-Cooper-Schrieffer (BCS) theory (19) was proven to be the correct description, and it remains one of the most successful theories in physics to date. The two basic ingredients of the BCS theory are (i) an “effective” attraction between the electrons and (ii) pairing of electrons into bosonic “Cooper pairs” (Fig. 2A). The Cooper pairs then form a Bose-Einstein condensate in the superconducting state. However, according to

is a source of attractive interaction between the quasiparticles; according to the BCS theory, it is the phonons.

To give the reader a rough idea of how this works, we consider two helium atoms. When the atoms do not overlap, their interaction is very weak and attractive: weak because the He atoms are neutral so the Coulomb interaction between their nuclei and electrons cancel, and attractive because the presence of one atom virtually (temporarily) excites the other atom into a high-energy state possessing an electric dipole moment (Fig. 2D). The attractive force is a result of the interaction between the virtual dipole moments.

Similarly, phonons are the key virtual excitations that turn the repulsive Coulomb interaction

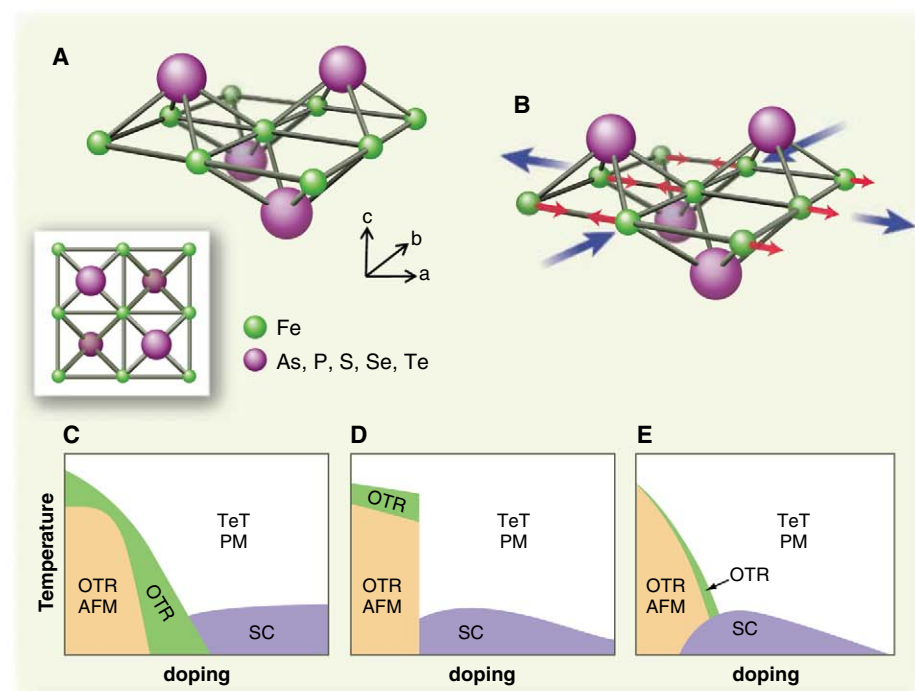


Fig. 1. (A) Structural motif of the FeSC. (Inset) Top view of the FeX trilayer. The triad (a , b , and c) demonstrates the three crystallographic directions. **(B)** The antiferromagnetic order of the stoichiometric iron-based materials. The red arrows represent the magnetic moments, and the blue arrows indicate the directions of structural distortion. **(C to E)** Schematic phase diagrams for three families of Fe-based compounds. OTR, orthorhombic; TeT, tetragonal crystal structure; AFM, antiferromagnetic; SC, superconducting; PM, paramagnetic phase.

Coulomb’s law, electrons should repel rather than attract one another. BCS theory addressed this apparent contradiction by invoking the attraction mediated by phonons (the quanta of atomic ion vibration) (Fig. 2, B and C). But how can the phonon-mediated attraction be strong enough to overcome the Coulomb repulsion? The answer to this question involves a bizarre property of metals: Despite the strong Coulomb repulsion, at low energy (or low temperature) a metal behaves as if its electrons were noninteracting entities called “quasiparticles.” Now all that is needed

into weak attraction in conventional materials such as mercury. However, for the high- T_c superconductors such as the cuprates and the FeSCs, there is a widely held belief (but not proof) that the electron-phonon interaction alone is not sufficient to overwhelm the residual Coulomb repulsion. This makes the electron pairing in these materials more perplexing. In fact, the residual Coulomb interaction in the cuprates is so strong that the energy is raised by a few electron volts (this is called the charge gap) when two electrons run into each other in the same crystal

Superconductivity

unit cell. This “Coulomb blockade” makes the stoichiometric cuprates “Mott insulators”: Each unit cell is occupied by a single electron because of the high energetic price required to double the occupancy. Virtual excitations above the charge gap generate an effective AF interaction. This interaction triggers antiferromagnetism in the undoped compounds. In doped compounds, it can serve as the attraction required for the formation of Cooper pairs, so long as the doped carriers do not bump into each other. Because FeSCs also have AF fluctuations (18), it is natural

the pair wave function to be s, d, etc. Scanning tunneling microscopy estimated the spatial extent of a Cooper pair in the *ab* plane (Fig. 1) to be $\sim 30 \text{ \AA}$ (23); the zero temperature London penetration depth (that is, the distance over which the magnetic field decays as a result of the Meissner effect) is $\sim 3000 \text{ \AA}$ (24). These parameters place FeSCs among strong “type II superconductors.” Aside from these, for conventional superconductors, T_c marks the formation of a Cooper pair. In contrast for the lightly doped cuprate superconductors, pre-formed Cooper pairs Bose-Einstein

starting from the lowest energy. If the system is a metal, there will be a closed equal-energy surface (or surfaces) called a Fermi surface(s) (FS) in the momentum space that separates the occupied and empty electron states. For a nonsuperconducting metal, promotion of an electron from beneath the FS to above can cost practically zero energy. This is no longer true for a superconductor: an energy gap develops on the FS. The energy gap is proportional to the absolute value of the so-called superconducting order parameter, which is associated with the quantum mechanical wave function of the Cooper pairs in momentum space.

Soon after the discovery of the FeSCs band structure, calculations based on what is known as density functional theory were carried out (25–27), showing that these materials have many bands and several disconnected FSs. For the highest- T_c (1111) compounds, these FSs are approximately disconnected cylinders (their cross sections are schematically shown in Fig. 3A). The red (or blue) lines mark the electronlike (or holelike) FSs, which expand (or contract) when extra electrons are injected. These cross sections are basically the FSs of the FeX trilayer.

For systems with multiple FSs, the scattering of an electron pair from one FS to another (Fig. 3B) is possible. In a generalization of the BCS theory to systems with two FSs (28), it was shown that superconductivity is possible even when the intra- and inter-FS pair scattering are both repulsive, as long as the latter dominates. Furthermore, under that condition, the order parameter will have an opposite sign on the two FSs.

Calculations of magnetic susceptibility (26, 27), showed that FeSCs have a tendency to order antiferromagnetically. The wave vectors associated with the spatial periodicity of the magnetic moments (Fig. 1B) coincide with those connecting the centers of the electron and hole FSs in Fig. 3A. On the basis of these observations, Mazin *et al.* (26) conjectured that AF spin fluctuations can induce the $s_{\pm}$ pairing. Here, $s_{\pm}$ refers to the fact that the order parameter is unchanged by the symmetry operations of the crystal, and $\pm$ refers to the sign change of the order parameter between the electron- and holelike FSs.

Many theoretical approaches based on the above itinerant electron point of view have been used to address the pairing problem in FeSC. For example, these include random phase approximation (RPA) (27, 29), fluctuation-exchange approximation (30), and renormalization group (RG) method (31–33). The conclusions yielded by different flavors of pairing theory depend critically on what electron-scattering processes are included. The RPA focuses on a subset of these processes and works best if the relevant virtual processes are known. In contrast, RG calculations (34) take into account all virtual two-electron scattering processes under the assumption that the interaction strength is weak compared with

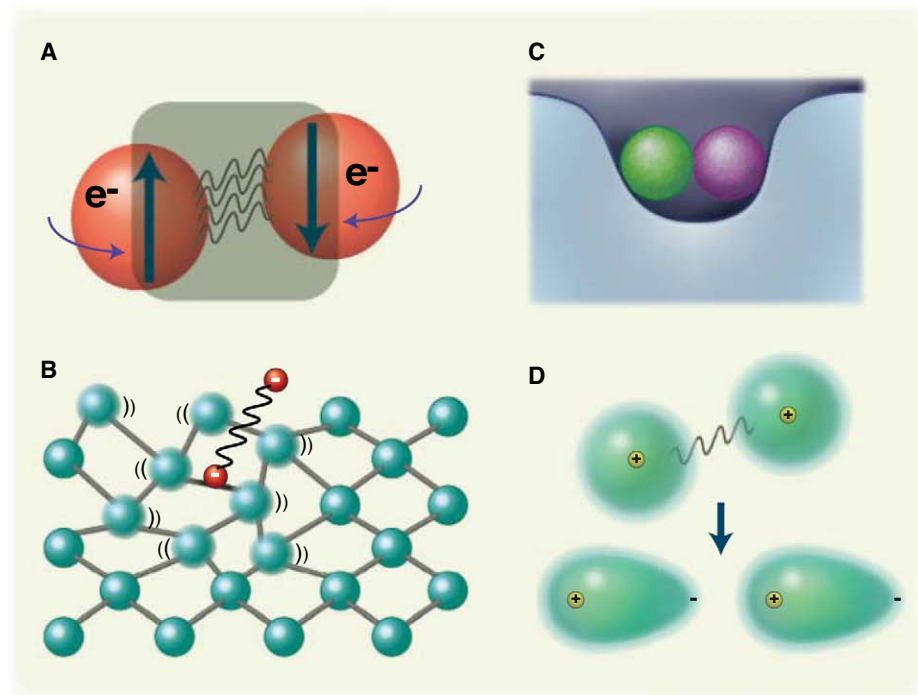


Fig. 2. (A) Schematic of a Cooper pair: a spin-up and spin-down (thick arrows) electron pair into a total spin-zero composite. In most superconductors, the average distance between the Cooper pairs is not small compared with the Cooper pair size. The wavy line represents the phonon-mediated attraction. (B) Phonon-mediated electron-electron attraction. When an electron (red circles) collides with the atomic lattice (green circles), it sets up a vibration. The second electron is affected by the vibration and, hence, “feels” the presence of the other electron and is attracted to it. (C) The waterbed analogy of the phonon-mediated attraction. An electron (marble) dents the lattice (water bed) and its effect is felt by another electron (marble). (D) The effective force between helium atoms. (Top) Two spherically symmetric He atoms approach each other. (Bottom) Both He atoms are virtually excited to high-energy states, which possess electric dipole moments and are attracted to each other.

to expect that they too possess some kind of effective AF interactions. However, as the undoped Fe-based materials do not have a charge gap, it is not clear at what energy scale such AF interaction would emerge.

Before we start considering the various pairing theories of FeSCs, let us review some of the established facts about the superconducting state. There is a consensus that Cooper pairs in almost all FeSCs have zero total spin angular momentum (20–22). Because the total wave function of a pair must be antisymmetric with respect to electron exchange, this restricts the orbital symmetry of

condense at T_c . Luan *et al.* (24) have shown that for FeSC it is the Cooper pair formation that controls T_c .

Pairing Theories

A good starting point for understanding a solid is to replace the electron-electron interaction by an averaged potential and assume that the electrons are otherwise noninteracting. Under this approximation one can calculate the energy-momentum relation (i.e., the band structure) of the electrons in the solid. According to Pauli’s exclusion principle, electrons will occupy the momentum states

the kinetic energy bandwidth. In the language of the aforementioned He atom analogy, a RG analysis keeps track of the effects of all virtual excitations above a “cutoff energy” and calculates the change of the effective interaction as the cutoff energy is lowered. A particular realization of the RG method, functional RG, further combines these features with the band structure in a single framework (35). Functional RG is capable of surveying all electronic instabilities on equal footing; hence, it is the least biased method (34, 36). Despite the diversity in the methods used, all of these theories come to the conclusion that when the antiferromagnetism is suppressed, $s_{\pm}$ pairing is favored. In addition, they agree that the most important virtual excitations that trigger the $s_{\pm}$ pairing are the “AF spin fluctuations,” namely a dynamic version of antiferromagnetism. Interestingly, a thorough analysis of the functional RG results reveals that, in addition to the antiferromagnetism and superconductivity, the FeSCs have a propensity to FS distortion and charge-density wave order as well (Fig. 4) (37).

Most of the theoretical methods mentioned above work best when the electron-electron interaction is weak. Through measurement of the electron effective mass, it has been concluded that FeSCs are intermediate coupling materials (17). Hence, it is worthwhile to look at the pairing problem from the opposite, strong-coupling limit. In this approach, one posits that FeSCs are equivalent to doped Mott insulators (15, 38, 39) with an effective nearest- and second-neighbor antiferromagnetic exchange interaction generated by the virtual excitations above the assumed charge gap. In this way, antiferromagnetism (15, 38) and $s_{\pm}$ pairing (38, 39) are also found.

Finally, a variational Monte Carlo calculation that treats the FeSCs as an intermediate correlated metal has been done recently (40). The results are qualitatively consistent with the functional RG predictions.

At this point, we would like to contrast the cuprates and the FeSC. They are similar in the sense that both possess strong effective AF interaction at low energies. This interaction is responsible for antiferromagnetism and likely Cooper pairing as well. However, for the cuprates, the states below the charge gap are subjected to the constraint that there can be no more than one electron per unit cell. This constraint is believed to be responsible for many unusual properties of the lightly doped cuprates. These include the high

pairing temperature and low Cooper-pair condensation temperature. Fe-based compounds do not have the occupation constraint. This is probably why the order of these two temperatures are reversed in FeSC (24).

The Pairing Symmetry

Presently, two types of experiments exist that bear more direct information concerning $s_{\pm}$ pairing. One of them is the detection of neutron resonance mode in the superconducting state. As suggested in (41, 42), if the pairing symmetry is

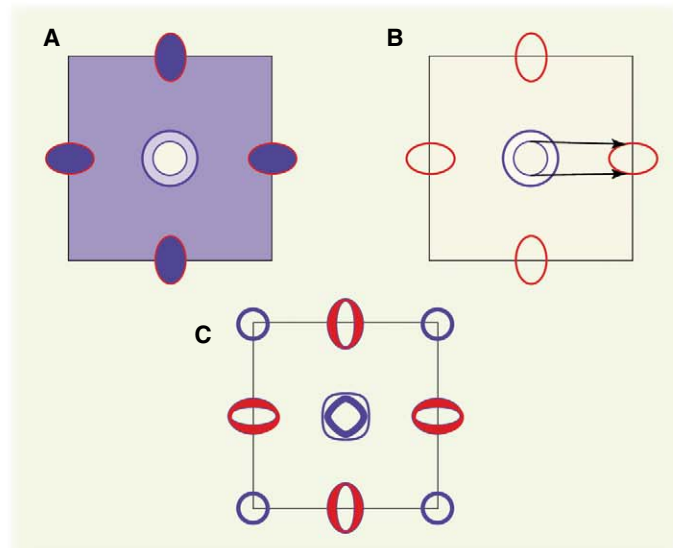


Fig. 3. (A) Electron- and holelike FSs of FeX trilayers are indicated by the red and blue curved lines, respectively. We use the “unfolded zone” representation corresponding to one Fe atom per unit cell. The color shading reflects the electron occupation at each momentum point (increasing from white to blue). (B) Inter-FS pair scattering. (C) Schematic representation of the superconducting order parameter obtained from several weak coupling theories. The width of the blue and red bands represents the magnitude of the order parameter. The color represents the sign: blue, positive; red, negative. The order parameter associated with four extra-small holelike FSs at the corner of the Brillouin zone is also shown here. These extra hole FSs are present in materials with large hole doping.

$s_{\pm}$, one expects a neutron resonance at the antiferromagnetic ordering wave vector upon entering the superconducting state; this peak was first observed in (43). The second experiment is based on the quasiparticle interference of scanning tunneling spectroscopy. If the superconducting order parameter has opposite signs on electron and hole FSs, it will impact the elastic scattering of quasiparticles by impurities (44, 45). Specifically, nonmagnetic impurities will scatter quasiparticles from hole to electron FSs (and vice versa), but not from hole to hole or from electron to electron FSs. If the impurities are magnetic, the reverse is true. This behavior is observed in a recent Fourier-transform scanning tunneling spectroscopy experiment for the $\text{Fe}_{1+x}(\text{Se},\text{Te})$ compounds (46); the relative strength of magnetic versus nonmagnetic scattering is controlled by a magnetic field.

In other phase-sensitive experiments, a scanning superconducting quantum interference de-

vice measurement was performed on polycrystalline $\text{NdFeAsO}_{1-x}\text{F}_x$ and found no evidence of half-integer flux quantum (47). Zhang *et al.* found a substantial c -axis Josephson tunneling between Pb and $\text{Ba}_{1-x}\text{K}_x\text{Fe}_2\text{As}_2$ (48). These two results make nonzero angular-momentum pairing, such as d-wave, unlikely. In the third experiment, electromagnetic pulse-induced half-flux quantum jumps were observed in a loop formed by Nb and polycrystalline $\text{NdFeAsO}_{0.88}\text{F}_{0.12}$ (49). This suggests that there are “ π junctions” along the current path resulting from Cooper pairs tunneling between opposite-sign superconducting regions. Taken together, the three experiments are consistent with the proposed $s_{\pm}$ pairing.

The simplest $s_{\pm}$ pairing predicts a nodeless superconducting gap. However, the order parameter can, in principle, vary substantially around different FSs as suggested in (27, 29, 31). An illustration of the variation is given in Fig. 3C. In certain cases, this variation can be so strong that “accidental” nodes are produced (29, 50). A node in the superconducting gap is the location on the FS where the energy gap vanishes. Measurements of the superconducting gap in several different FeSCs by angle-resolved photoemission spectroscopy have revealed a more-or-less uniform gap on all observable FSs (51, 52). However, there is also experimental evidence (for example, from nuclear magnetic resonance and penetration-depth experiments) for the existence of gap nodes in some materials (20, 53–56). Recently a direct comparison between the results of the surface-sensitive scanning tunneling spectroscopy measurement (46) and a bulk-sensitive,

angle-resolved specific-heat measurement (57) for $\text{Fe}_{1+x}(\text{Se},\text{Te})$ became available. Whereas the tunneling spectra of Hanaguri *et al.* (46) indicate a nodeless superconductor, the result of Zeng *et al.* (57) shows evidence for either nodes or minima of the energy gap. Chubukov *et al.* and Vorontsov *et al.* have argued that these results are consistent so long as the nodeless gap exhibits angular variation (such as shown in Fig. 3C) (58, 59). Thus, with a dose of cautious optimism, it seems that substantial evidence supports the notion that at least one FeSC shows $s_{\pm}$ pairing. However, this does not mean that every FeSC has to do so.

Some Nonuniversal Properties of FeSCs

Many important properties of the Fe-based compounds vary from system to system. For example, there is clear evidence that some of the FeSCs do have nodes (55), whereas others seem to have a

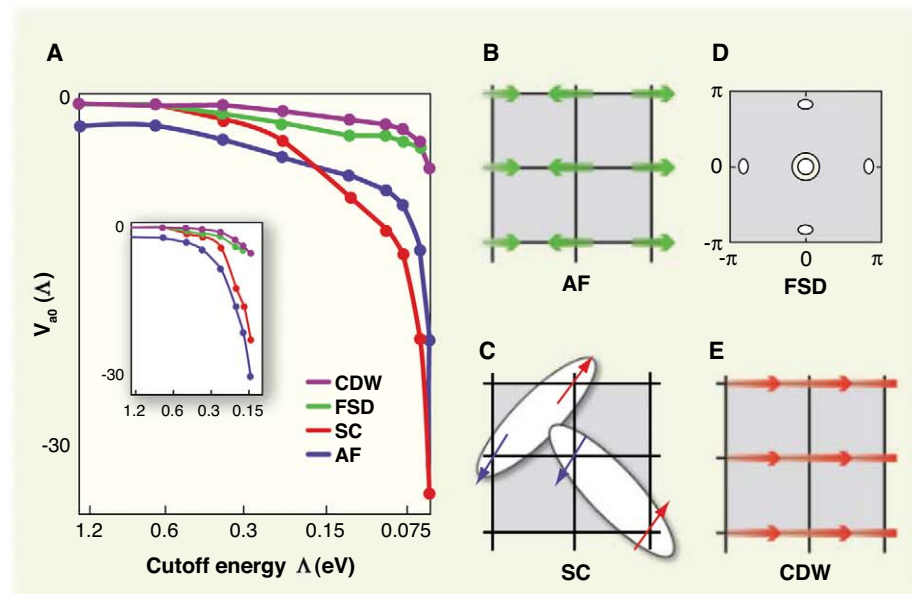


Fig. 4. (A) Evolution of the antiferromagnetic (AF), superconducting (SC), Fermi surface distortion (FSD), and charge density wave (CDW) ordering tendencies with the energy cutoff (or the temperature) of the RG calculation (37) in a superconducting sample (main panel) and an AF sample (inset). The vertical axis is a measure of the degree of instability: more negative values imply stronger instability. Schematics of the four ordered patterns are shown. (B) AF order. The green arrows represent the magnetic moments. (C) Superconductivity. The oblong shape denotes a Cooper pair. (D) FS distortion. The circles are all holelike FSs. (E) Charge density order. The red arrows represent the direction of bond current.

full gap (46, 51, 52). Another example is the topology of the phase diagram. Figure 1, C to E, shows the schematic representation of the phase diagrams of $\text{CeFeAsO}_{1-x}\text{F}_x$ (12), $\text{LaFeAsO}_{1-x}\text{F}_x$ (60), and $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$ (61), respectively. In $\text{CeFeAsO}_{1-x}\text{F}_x$ (Fig. 1C) and $\text{LaFeAsO}_{1-x}\text{F}_x$ (Fig. 1D), the antiferromagnetic phase does not overlap with the superconducting one. In $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$ (Fig. 1E), on the other hand, the two phases overlap. Whether antiferromagnetism homogeneously coexists with superconductivity in the latter case or whether there is local phase separation is currently under debate. Moreover, the AF phase of $\text{LaFeAsO}_{1-x}\text{F}_x$ terminates very abruptly, but for $\text{CeFeAsO}_{1-x}\text{F}_x$ and $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$, it disappears continuously. The nature of the AF order can also be material-dependent. For example, the AF pattern in Fe_{1-x}Te is different from that shown in Fig. 1B by 45° , and the period is twice as long (62); moreover, upon $\text{Te} \rightarrow \text{Se}$ substitution, the magnetic order becomes incommensurate (62). It is commonly felt that these nonuniversal behaviors are intrinsic rather than caused by poor sample quality.

It is very tempting to conclude that a strong effective AF interaction is responsible for the high T_c of both FeSCs and the cuprates (63). If so, maximizing the ratio between the AF interaction strength and the carrier's kinetic energy bandwidth, while avoiding antiferromagnetic and other competing orders, is the key to high- T_c superconductivity. However, the often controversial

history of the exploration of superconductivity in the cuprates gives us plenty of reason for caution.

Aside from what has already been discussed, there are many more unanswered questions. For example, is the $s_{\pm}$ pairing universal to all FeSCs? Can $s_{\pm}$ survive the relatively strong disorder in many FeSCs? What is the pairing symmetry in the newly discovered $\text{A}_x\text{Fe}_{2-y}\text{Se}_2$? Why do certain delicate structural parameters, such as the As-Fe-As bond angle, affect T_c so sensitively? Why is there a persistent disagreement between the angle-resolved photoemission result and other bulk-sensitive probes concerning the anisotropy in the gap function? Does the electron-lattice interaction play an important role in electron pairing? What is special about iron? Last but not least, where do we find other higher T_c superconductors? We look to the future for the answers to these and other important questions.

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Kepler Detected Gravity-Mode Period Spacings in a Red Giant Star

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Red giants are evolved stars, representing the future Sun, and were recently discovered to oscillate in acoustic modes similar to those found in the Sun (1, 2). These modes are stochastically excited by convective motions in the star's outer layers and obey frequency spacing laws understood in terms of the theory of stellar oscillations (3, 4). These frequency patterns are used to derive the basic physical stellar parameters, such as the mass and radius, with unprecedented accuracy.

Unlike pure acoustic modes, mixed modes probe deeply into the interiors of stars, allowing the derivation of stellar core properties, such as the local density structure, the chemical composition gradient, and the near-core angular momentum, that would otherwise remain inaccessible.

We detected mixed modes in the red-giant star KIC 6928997 (Kepler Input Catalog) on the basis of 320 days of observations with the Kepler satellite (5). The oscillation spectrum of KIC 6928997 (Fig. 1) (6) deviates from the pattern expected for pure, short-lived acoustic modes (fig. S1). This indicates the presence of mixed

modes that have the character of a gravity mode in the core region and of an acoustic mode in the envelope of the star. From a theoretical perspective, modes with most of their energy in the core will not be observed because they get trapped there. However, in the case of dipole ($\ell = 1$) modes, the trapping is less efficient, and some of these mixed modes probing the core could reach substantial amplitudes at the surface (3, 4). The observed power spectrum of KIC 6928997 is in agreement with predicted spectra of such densely populated core-probing mixed modes. The lifetimes of these mixed modes must be longer than those of pure acoustic modes (1), because their mode broadening from damping is not yet fully resolved in the power spectrum (6). This is consistent with the predictions (3, 4).

The observed period spacings of the mixed modes of KIC 6928997, that is, the distance in period between modes of consecutive radial order, are shown in Fig. 1B. The spacings of dipole modes lead to a characteristic shape, which is understood from theory as a consequence of the interaction between the acoustic and gravity

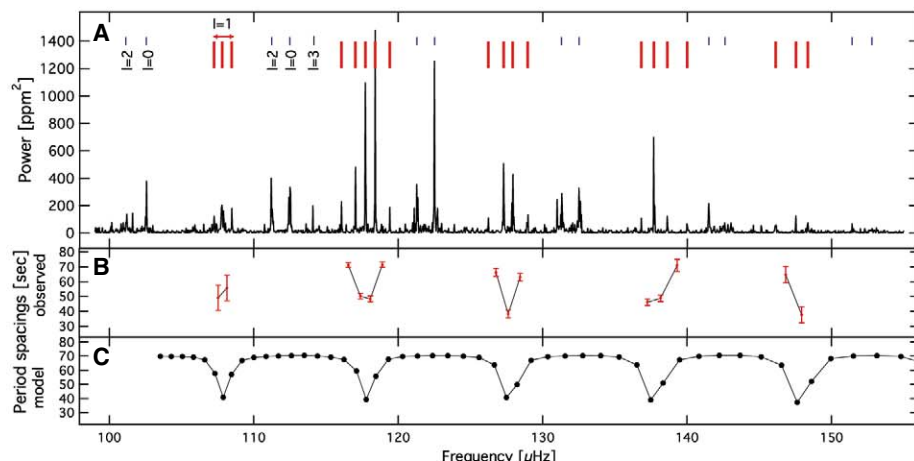


Fig. 1. (A) Oscillation spectrum and (B) corresponding period spacings of mixed $\ell = 1$ modes for the red-giant star KIC 6928997 as observed by the Kepler satellite. The position of the radial and quadrupole acoustic modes ($\ell = 0$ and 2) is indicated in blue, $\ell = 3$ in black, and the fine structure of the mixed modes in red ticks. ppm, parts per million; error bars in (B) indicate the uncertainty of the observed period spacings. (C) Adiabatic period separations for $\ell = 1$ modes derived from a stellar model similar to KIC 6928997 (6).

mode cavities for such modes (fig. S2) and is a key indicator of the core properties. There is a good qualitative agreement between our observations (Fig. 1B) and the pattern of theoretically predicted spacings for an appropriate stellar model (Fig. 1C). The observed period spacing, along with its detected characteristic structure, provide a lower bound for the constant period spacing, which is directly dependent on the density contrast between the core region and the convective envelope.

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Nanometer-Thick Equilibrium Films: The Interface Between Thermodynamics and Atomistics

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Nanometer-thick films at interfaces and surfaces exist in various materials and can substantially influence their properties. Whether these films are an equilibrium or transient state is debated. To address this question, we equilibrated 1.2-nanometer-thick films at gold-sapphire interfaces in the presence of anorthite glass and measured the solid-solid interface energy. The equilibrated film significantly reduced the interfacial energy and could be described by the Gibbs adsorption isotherm expanded to include structure in addition to chemical excess. Unlike artificially made conventional thin films, these films do not break up during equilibration and offer an alternative design criterion for thin-film technology. These results demonstrate that nanometer-thick films at interfaces and surfaces can be an equilibrium state and included in phase diagrams with dedicated tie-lines.

Films of ~1-nm thickness at grain boundaries (GBs) in polycrystalline materials, phase boundaries, and free surfaces are ubiquitous. They have been identified at GBs in ceramics, where they limit high-temperature mechanical properties and influence grain growth during sintering, and can be used to engineer functional properties (1–6). The same type of nanometer-thick film has been identified at the surface of oxides (7) and polymers (8), including the surface of ice, where a thin (nanometer range) film of water forms, providing the lubricant for ice-skating (9), and causes the shifting of petroleum pipes located in northern regions (10). More recently, such films have been identified at interfaces between metals and oxides (11–14) and at GBs in metallic alloys (15, 16). One of the main characteristics of these films is that their thickness and average composition are constant along a particular interface or surface, and they are not considered a bulk phase.

A fundamental question is whether these nanometer length-scale films, which are often called intergranular films (IGFs), are an equilibrium interface state, a wetting film (which is a bulk phase), or a transient phenomenon due to impurities, mass transport, or oxidation (Fig. 1). If they are indeed an equilibrium interface state, then IGFs can potentially be mapped onto bulk phase diagrams as tie-lines, which define the equilibrium state of (two-dimensional) surfaces and interfaces (17, 18). Here, we present an experimental approach to measure interface energy with and

without IGFs and show that 1.2-nm-thick IGFs at gold-sapphire interfaces are stable interface states associated with a decrease in excess energy.

Equilibration of gold particles on sapphire in the presence of glass. Gold films (60 nm thick) deposited on sapphire (α -Al₂O₃) basal plane substrates were annealed in air at 1100°C for 2 hours, after the substrates were first partially coated with anorthite glass (CaO-2SiO₂-Al₂O₃) droplets (details in Materials and Methods). Anorthite is a source of the elements that are often found in

IGFs at alumina grain boundaries (4) and metal-alumina interfaces (11–14), and the presence of bulk anorthite specifically defines the chemical potentials of these elements in our experiments. Due to the finite contact angles of gold on anorthite and sapphire, during thermal annealing the gold film broke up into small particles dispersed on the substrate (Fig. 2A) (14). Cross-section transmission electron microscopy (TEM) specimens were prepared using a dual-beam focused ion beam (FIB) system (Fig. 2B) (19) and examined by aberration-corrected high-resolution TEM (HRTEM). The results were compared to measurements from specimens prepared by the same process but equilibrated without anorthite glass (20).

Morphological characterization of the intergranular film. The interface between gold and sapphire examined by HRTEM showed the presence of a 1.2-nm-thick IGF (Fig. 2C). The film composition was found to include Ca and Si (14) but at a different concentration than in anorthite, which together with the finite and constant thickness indicated that this was not a wetting film (Fig. 1). If it were a wetting film (Fig. 1B), the film thickness would have increased as a function of increasing anorthite glass volume in the system, which was not the case. With an IGF, a preferred orientation of the (111) gold plane was found parallel to the sapphire (0001) surface, while no low-index orientation relationship existed between the gold particles and the sapphire (table S1). Without an IGF, both (111) and (200) gold preferred orientations were found parallel to (0001) sapphire, with defined low-index orientation relationships

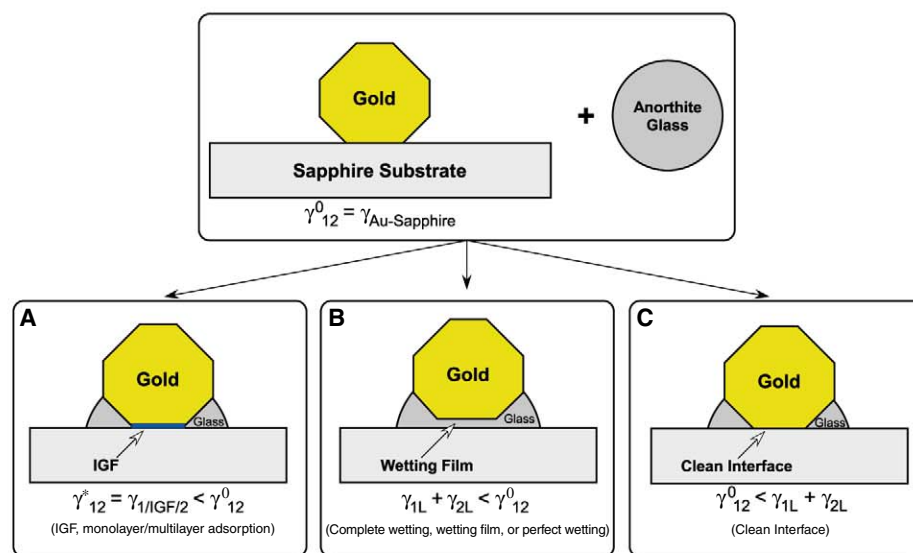


Fig. 1. Schematic drawings of an interface, formed when two bulk phases (gold and sapphire) are equilibrated in the presence of a third phase (bulk anorthite glass). Although the final geometric configuration will minimize the total interface and surface energies (via contact and dihedral angles), the interface between the gold and sapphire can adopt one of the following configurations: (A) An intergranular film forms at the interface, which has a lower interface energy than the original gold-sapphire interface; (B) a wetting film forms between the gold and sapphire, resulting in two interfaces (1L and 2L) between the sapphire and glass and between the gold and glass; or (C) a "clean" interface forms having an energy equivalent to that of the original configuration.

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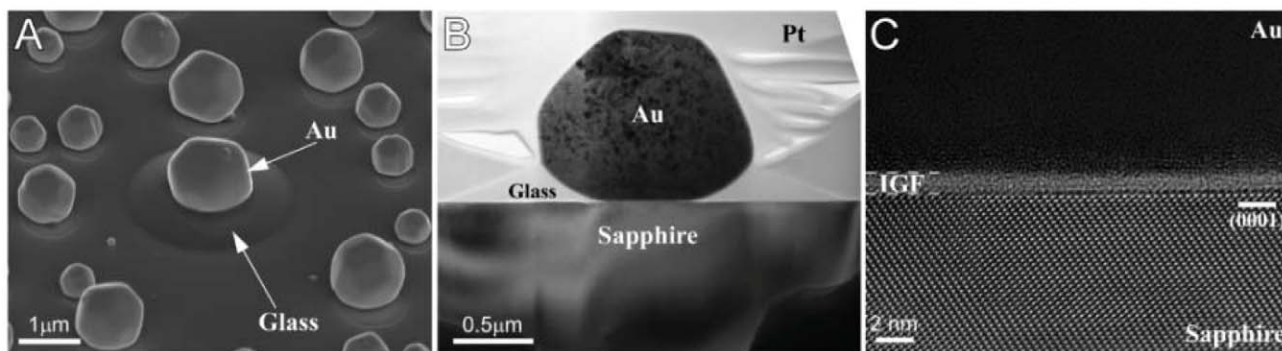


Fig. 2. (A) Secondary electron scanning electron micrograph (acquired with the dual-beam FIB) showing a single gold particle located on an anorthite drop. (B) Bright-field TEM micrograph of a cross-section TEM specimen prepared from a gold particle on an anorthite drop such as in (A). This specimen was prepared with the FIB “lift out” technique. The contrast above the gold is

from the protective Pt coating that was deposited before the FIB “lift-out” process. (C) Aberration-corrected HRTEM micrograph ($C_s \sim -5 \mu\text{m}$) of an equilibrated interface between a gold particle and a basal plane sapphire substrate. A $\sim 1\text{-nm}$ -thick IGF has equilibrated at the interface. The micrograph was recorded after orienting the sapphire in the $[1210]$ zone axis.

(20, 21). This indicated that the IGF produced a decrease in the in-plane anisotropy of the gold-sapphire interface energy.

Interface energy measurements. One advantage of equilibrating metal particles in contact with the sapphire substrate at thermodynamic coexistence with bulk anorthite is that the gold-sapphire interface energy can be determined in the presence of an IGF, at controlled chemical potentials. Figure 3 presents a schematic illustration of the geometric configuration of a gold particle that partially resides inside an anorthite droplet on the sapphire substrate. All the relevant surface and interface energies are indicated in Fig. 3, and the experimental contact and dihedral angles are defined in the micrographs presented in Figs. 4 and 5.

From dihedral and contact angle analysis (see table S2 and “Determination of interface energy” in the SOM), a reduction in interface energy of $\sim 190 \text{ mJ/m}^2$ occurs in the presence of the IGF at the gold-sapphire interface, compared to the same interface without an IGF (20). Given the potential impurity level of the starting materials used for the analysis conducted in (20), monolayer or sub-monolayer interface adsorption may have occurred, indicating that the interface energy of hypothetically pure gold-sapphire interfaces (without an IGF) is even higher than that reported. An even larger reduction in interface energy is expected if adsorption of the glass constituents to the sapphire and/or gold surfaces has occurred, providing experimental proof for the reduction of the gold-sapphire interface energy in the presence of an IGF. The x-ray diffraction results (table S1) further corroborate these findings, demonstrating a reduction in in-plane interface anisotropy due to the presence of an IGF (resulting in a preferred orientation instead of a low-index orientation relationship, as was mentioned before).

Intergranular films (complexions) and the Gibbs adsorption isotherm versus wetting films. Since the discovery of IGFs, their origin and stability have been debated. It was assumed that IGFs, in a manner similar to that of wetting films, reduce the interfacial energy of a system. Clarke *et al.*

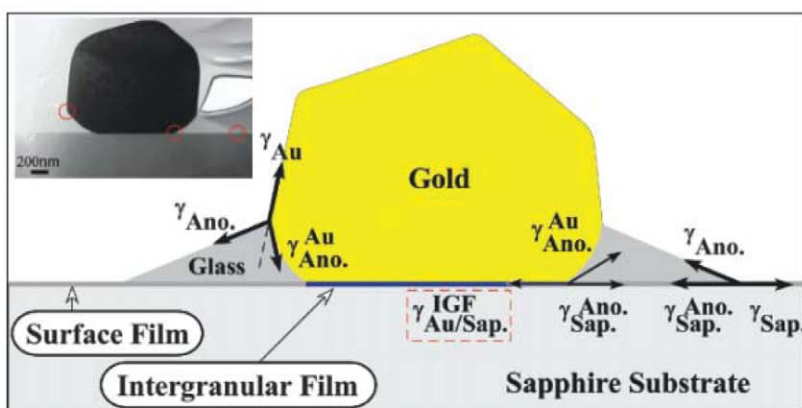


Fig. 3. Schematic drawing of the geometric configuration of the system with a gold particle partially immersed in an anorthite drop. All the relevant surface and interface energies are indicated. The inset (upper left corner) presents a TEM micrograph of a real gold particle on an anorthite droplet, on the sapphire substrate.

(22, 23) extended the wetting film analogy to IGFs by defining two film-crystal interfaces instead of considering only one interface that includes the IGF, as was done in this study. Treating the IGF as a single interface is more correct, because the IGF is not a bulk phase but rather an interface state (17, 24), so its properties differ from those of a bulk wetting film. The reduction in energy due to the existence of IGFs was predicted from general experimental observations and theory (17, 22, 25, 26), in that IGFs do not exist at all GBs, but rather exist only at high-angle (high-energy) GBs, causing their energy to be reduced to that of special (low-energy) and/or low-angle GBs (2, 22). In addition, in an extensive study conducted on surface films, it was predicted that the reduction in energy is necessary for stabilization (27, 28). The reduction in interface energy measured in this work confirms these studies.

Dillon *et al.* indirectly compared the relative energies of GBs containing different interface states [called “complexions” by the authors (18)] by correlating GB dihedral angles (29) with GB mobility (18). They found that in some cases, a reduction in energy occurred due to a change of

complexion that was inferred from GB mobility measurements. Although it is difficult to compare the relative values averaged for different GBs with the absolute values determined in the present study, the prior work provides evidence that the phenomena reported here occur for a wide variety of complex GB geometries. Yoshiya *et al.* showed from molecular dynamics simulations that the IGF is indeed energetically favorable at Si_3N_4 twist boundaries, where the excess free energy was correlated with the segregation of atoms with dangling bonds at the interface (30).

Although various models have been used to address IGFs, they can also be directly defined via the Gibbs adsorption isotherm (31)

$$d\gamma = -\sum_i \Gamma_i d\mu_i \quad (1)$$

where a surface (or interfacial) energy (γ) is reduced by the presence of (surface) interfacial excess (Γ) and a change in chemical potential (μ). The key for applying this isotherm to IGFs is to determine the dividing surface (interface) of Gibbs, which is a mathematical plane between the two phases that contains the interfacial excess. This excess is not necessarily located on one atomic

plane but can be spread over several atomic planes, starting from part of a monolayer and extending up to (but not including) a wetting film. Most of the analytical models (and related experiments) that were developed to obtain the change in surface energy due to adsorption usually ignore the interface structure and consider only the interfacial chemical excess (number of atoms per area, or number of monolayers) (32). The local atomistic structure must be included, because IGFs are expected to have some varying order adjacent to the crystals (33, 34) and the degree of order is expected to influence the energy (35). Indeed, ordering in liquids adjacent to solids and reconstructed surfaces can be defined as types of complexions (36). We have also experimentally confirmed that the IGFs in this study are partially ordered (37), and thus the excess (Γ) used to define an IGF must include a description of both the interface chemistry and structure, which can then be correlated with the decrease in interface energy via the Gibbs isotherm.

Diffuse interfaces, van der Waals, and image forces. Since IGFs are an equilibrium state, complexion diagrams (similar to phase diagrams) can be constructed to predict interfacial structure and

chemistry, by using phase-field and diffuse interface models (25, 38, 39) [the latter partially based on Cahn's critical point wetting theory (40)]. In these models, in contrast to the approach of Gibbs and Cahn, a function is used to describe the misorientation and structural order of an interface (41). One prediction from these models is that partially disordered interface states are more likely to be stable at high-energy GBs than at low-energy GBs [identical to the experimental observations (22)].

Force-balance models, based on a balance between various attractive forces (e.g., van der Waals forces) and repulsive forces (e.g., steric forces), have been widely used to describe interfaces with an IGF (42). Values of the Hamaker coefficient can be used to estimate attractive London (van der Waals) forces at grain boundaries and interfaces (43). Lipkin *et al.* developed a model to assess the contribution of the van der Waals forces to the thermodynamic work of adhesion (W_{ad}) at metal-alumina interfaces without an IGF and calculated a contribution of 330 to 560 mJ/m² to the value of W_{ad} (44). From comparison to experimental results, they concluded that for gold-alumina interfaces, van der Waals forces are an important contribution to W_{ad} . However, if we perform the same calculation for the attractive London dispersion forces at a gold-sapphire interface with an IGF, we reach the opposite conclusion. Our measured W_{ad} (640 mJ/m²) is three orders of magnitude higher than the van der Waals contribution (0.94 mJ/m²), when we use a Hamaker coefficient calculated for the gold-sapphire interface containing an IGF (14). Therefore, we conclude that the main IGF contribution to the reduction in interface energy at gold-sapphire interfaces is not a simple van der Waals interaction.

Johnston and Finnis demonstrated that image forces should be considered for the case of metal-oxide interfaces containing an IGF, based on a classical density functional model used to calculate the electrostatic and entropic forces between two planar surfaces separated by a thin (~1-nm) glass film containing a distribution of counterions (45). The model was applied to the copper-sapphire interface with an IGF, on the basis of the experimental results for this interface (11). It was found that the image interaction force reduces the in-

terface energy by ~100 mJ/m², and it dominates over the van der Waals contribution (which was estimated to be 0.149 mJ/m²). The decrease in interface energy due to image forces is of the same order of magnitude as the experimental decrease in interface energy measured in the present study (~190 mJ/m²). Therefore, we conclude that image forces are a dominating attractive force at gold-sapphire interfaces containing an IGF.

We have shown here that equilibrating a two-phase system (gold and sapphire) in the presence of a third phase (anorthite) changes the chemical potentials, resulting in Gibbsian adsorption in the form of an IGF. In addition, the IGF has both a specific average composition and structure, which fits with the definition of a complexion (whereby interfaces can adopt states that have distinct interfacial structure and/or composition profiles, correlated with local minima in free energy). Furthermore, this finding can be used to develop grain boundary/interface complexion diagrams (using diffuse interface models). The diagrams may be used to predict the most stable structure and composition, and in combination with experiments, tailor materials with specific macroscopic properties.

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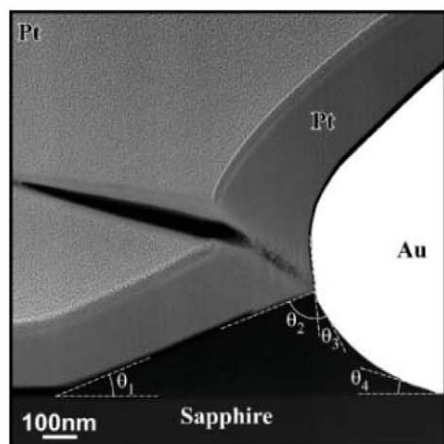


Fig. 4. Dark-field scanning TEM micrograph of the triple junction region, from which the interface energies were measured. All the angles that were used in the measurements are indicated on the micrograph.

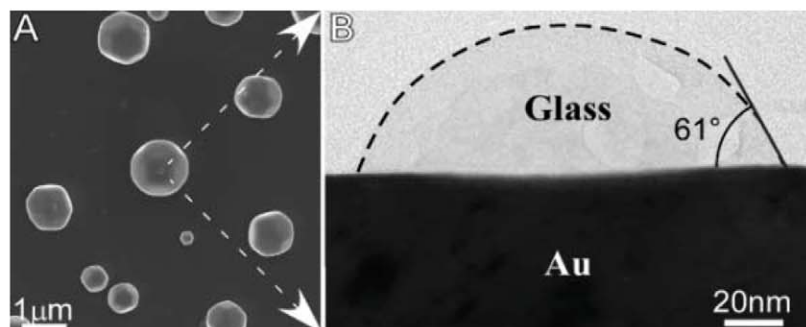


Fig. 5. (A) An example of a small glass droplet on a gold particle from which the glass-gold contact angle was determined. A cross-section TEM specimen was prepared from the particle and droplet by FIB. (B) A TEM micrograph of the droplet on the Au (100) facet.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6026/206/DC1

Materials and Methods

Tables S1 and S2

References

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Ribozyme-Catalyzed Transcription of an Active Ribozyme

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A critical event in the origin of life is thought to have been the emergence of an RNA molecule capable of replicating a primordial RNA “genome.” Here we describe the evolution and engineering of an RNA polymerase ribozyme capable of synthesizing RNAs of up to 95 nucleotides in length. To overcome its sequence dependence, we recombined traits evolved separately in different ribozyme lineages. This yielded a more general polymerase ribozyme that was able to synthesize a wider spectrum of RNA sequences, as we demonstrate by the accurate synthesis of an enzymatically active RNA, a hammerhead endonuclease ribozyme. This recapitulates a central aspect of an RNA-based genetic system: the RNA-catalyzed synthesis of an active ribozyme from an RNA template.

An earlier, simpler biology might have relied on RNA for both heredity and metabolism. Evidence for such an “RNA world” (1) preceding modern life includes the central catalytic and informational roles of RNA in splicing, gene expression, and translation (2–4), as well as the versatility of RNA in forming specific receptors and catalysts (5, 6). Organisms of the putative RNA world would have required an RNA polymerase ribozyme for both RNA-based heredity and the expression of “RNA genes.” Although the ancestral replicase appears to have been lost, key functional aspects of RNA-catalyzed RNA replication can be studied “by proxy” with the use of modern ribozymes generated by in vitro selection, such as the R18 RNA polymerase ribozyme (Fig. 1A) (7). R18 was isolated from a random sequence pool by in vitro evolution and stepwise engineering of the initial class I ligase ribozyme (8–10). Although R18 is a general RNA polymerase, its activity is both sequence-dependent and limited to transcribing stretches of RNA up to 14 nucleotides (nt) long on a favorable RNA template (7). Starting from R18, we have used both RNA evolution and engineering to generate new RNA polymerase ribozymes with improved polymerase activity and sequence generality (11).

Compartmentalized bead-tagging. Strategies for the in vitro selection of catalytically active

RNAs commonly involve the acquisition (or loss) of a capture tag for recovery (12, 13). This restricts direct selection for more advanced enzymatic properties, such as multiturnover catalysis. Directed evolution of RNA polymerase ribozymes is complicated further by their poor primer extension efficiency: Substantial extension of a single primer remains a sporadic event, which reduces the effective size of polymerase ribozyme libraries. Furthermore, ribozyme repertoires must be transcribed by, for example, T7 RNA polymerase, which outperforms likely ribozyme polymerase candidates and must therefore be absent during the selective step. Finally, a sensitive and sequence-specific detection strategy is required to distinguish between simple nucleotide transferase and genuine templated polymerase activities.

We developed a selection strategy, termed compartmentalized bead-tagging (CBT), that harnesses water-in-oil emulsion technology (14) to link ribozyme genes to thousands of copies of the corresponding ribozyme via microbead display (fig. S1). CBT separates transcription from subsequent selective primer extension and allows the selection of clonal ribozyme populations rather than individual ribozyme molecules. The sampling of the aggregate activity of thousands of clonal ribozymes and a sensitive fluorescence signal amplification of extended primers by rolling circle amplification (RCA) (figs. S1c, S2, and S3) enable the discovery and isolation of polymerase ribozymes as a function of their primer extension capability. We validated CBT using model selections of R18 ribozyme genes spiked

into an excess of inactive R18 genes (R18i) (fig. S1b), which yielded up to a 10^5 -fold enrichment of active genes (fig. S1d).

Ribozyme RNA polymerase selection. We sought to improve ribozyme-primer-template interactions, known to be poor in the R18 ribozyme (7, 15, 16), by appending a random-sequence RNA domain to the 5'-end of R18, a region implicated in interactions with the primer-template duplex (17). A short “stem” RNA (table S1) that completes R18 by formation of a helix (P2) (7) was omitted from this selection as its functional relevance had been called into question (16).

CBT selection was applied to this library ($\sim 5 \times 10^7$ sequences) (table S2). We developed a microtiter plate-based assay (RPA, ribozyme polymerase plate assay) (fig. S4) to screen for clones of interest. After three rounds of CBT, we observed an increase in polyclonal ribozyme activity (fig. S5a) and, by RPA, identified one ribozyme (C19) with improved RNA polymerase activity among 22 clones screened (Fig. 2, A and B).

C19 differed from the parent R18 ribozyme by the presence of a new 5'-domain, as well as a single point mutation (G93A) (Fig. 1B). The G93A mutation was located in the P2 region of the ribozyme and compensated for the reduction in polymerase activity caused by the absence of the stem oligonucleotide. Indeed, the A93G back-mutation rendered C19 RNA polymerase activity stem-dependent (fig. S5b).

Secondary structure prediction of the new 5'-domain indicated that it comprised a 6-nt single-stranded sequence segment (ss_{C19}) at the 5' end, followed by a hairpin domain (HP_{C19}). ss_{C19} showed complementarity to the 5' end of the template (TI) that had been used for both selection and screening. Binding of ss_{C19} to the template was required for C19 activity, as templates containing successive mutations of the ss_{C19} complementary sequence showed a progressive loss in activity (Fig. 2C). Compensating mutations in ss_{C19} (to reconstitute a 6-nt hybridization site) restored activity, although not consistently to C19 levels. This suggests that ss_{C19} enhances C19 activity primarily (but not exclusively) by promoting recognition and binding of template downstream of the primer 3' end.

Long-range RNA synthesis. Although C19 showed improved polymerase activity on the selection template TI (Fig. 2B), the activity enhancement of C19 compared with R18 was much

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less pronounced on longer templates, where the ssC₁₉ binding site was located farther downstream of the primer (Fig. 2D). We speculated that C19 was structurally adapted to extending primers close to the ssC₁₉ binding site. We therefore engineered a series of 5'-domain variants of C19, where either single secondary-structure elements

[sequences upstream of ssC₁₉ (R10), ssC₁₉, and HP_{C19}], or combinations thereof, were omitted (fig. S6). One of these truncation variants, tC19 (Fig. 1C), lacking sequences upstream of ssC₁₉ and with HP_{C19} replaced by a four-residue adenine (A₄) spacer, showed improved RNA polymerase activity on the longer template TI-3 compared with

the parent C19 ribozyme (Fig. 2D), and extended 13% of primers by over 26 nt (table S4).

In the putative RNA world, both RNA replicases and replication templates would have been under selective pressure to coevolve toward maximum replication efficiency. We sought to mimic aspects of this adaptive process by evolving tem-

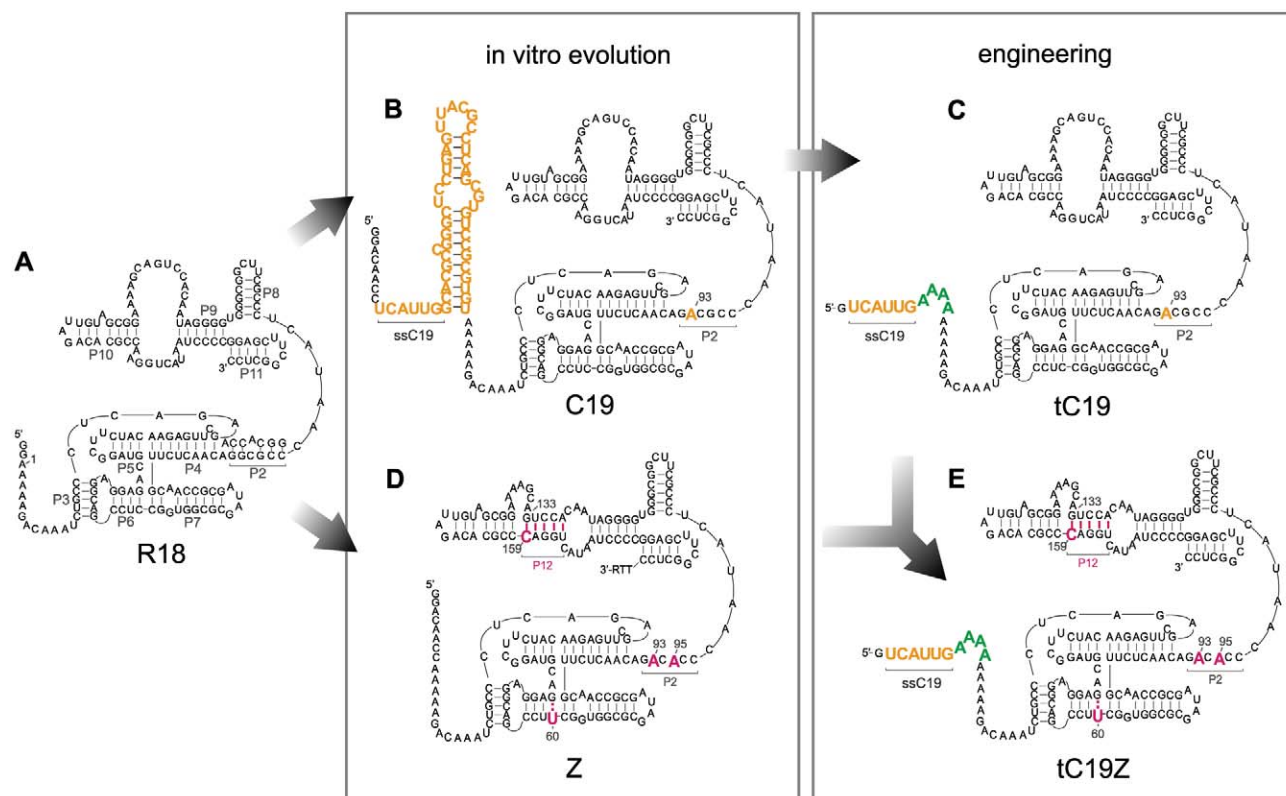


Fig. 1. Evolved and engineered ribozymes. Secondary structures of (A) R18 (7); (B) C19, as predicted by mfold (27); (C) tC19; and proposed secondary structures for (D) Z; and (E) tC19Z. Mutations isolated from the Z selection are depicted in

magenta, sequences isolated from the C19 selection in orange, and engineered residues in green. The A159C mutation is shown to reshape the processivity domain by stabilizing helix P12. [Run-through transcript, 3'-RTT (table S1).]

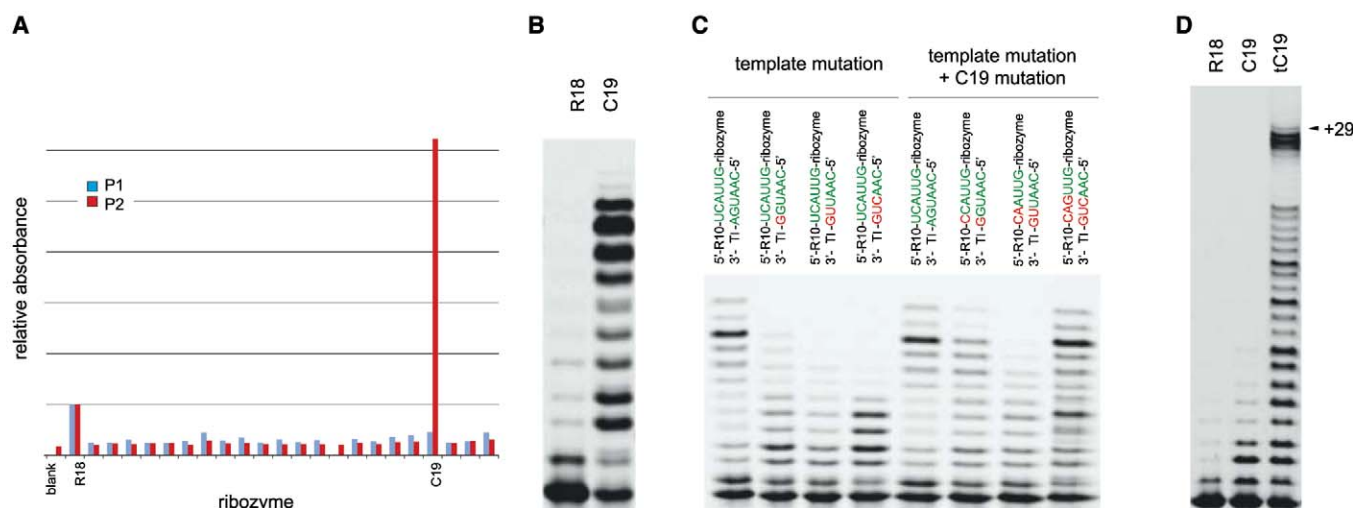


Fig. 2. Selection and engineering of C19. (A) RPA of clones from the N48 library selection after three rounds of CBT probed for basic activity (probe P1, blue) and more stringently (probe P2, red). Absorbance at 450 nm (as OD) is shown normalized to R18. (B) Primer extension activity of R18 and C19 ribozymes on the selection template TI. (C) Primer extension by C19 on templates

with successive point mutations (red) in the ssC₁₉ binding site ("template mutation"), and with compensating mutations (red) to reconstitute a 6-nt hybridization site) in C19 ("template mutation + C19 mutation"). ssC₁₉ and its respective binding site are depicted in green. (D) Primer extension activity of tC19 compared with the R18 and C19 ribozymes on template TI-3.

plates adapted to tC19 using the downstream ssC19 recognition site. We applied a template selection scheme (fig. S7) to a library of templates

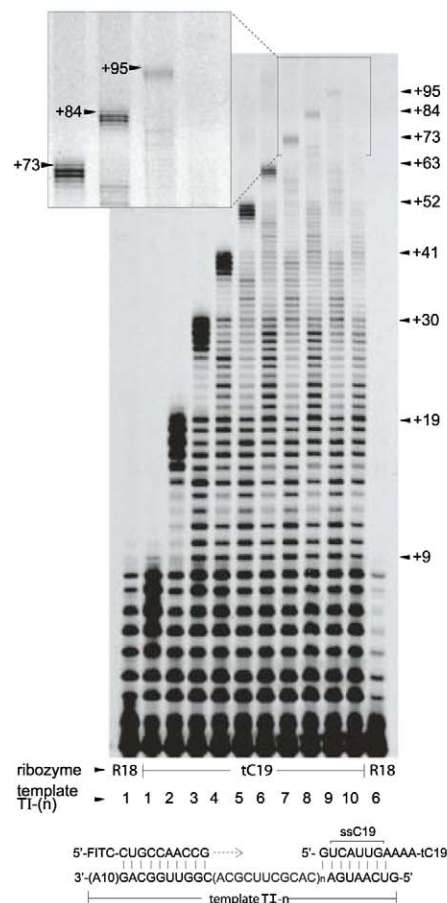


Fig. 3. Long-range RNA synthesis on engineered template series TI-n by the tC19 and R18 ribozymes (7 days). “n” indicates the number of repeats of the central 11-nt sequence between primer and ssC19 binding sites. The schematic depicts primer extension by tC19 on TI-n.

based on TI-3 (table S1). After two rounds of template selection, ~50% of sequences isolated were derived from a single template (TI-5), in which TI-3 had been extended by two additional repeats of the central 11-nt sequence. TI-5 could be transcribed to ≥ 47 nt by tC19, with 1.5% of primers extended beyond this point (table S4). We generated a series of templates based on the same design principles containing increasing numbers of the 11-nt repeat (TI-1 to TI-10) (table S1). On these templates, tC19 could extend primers by up to 95 nt (Fig. 3) with a fidelity (expressed as the probability of mutation per incorporated nucleotide) of 2.7×10^{-2} as determined by sequencing full-length extension products (fig. S8). Extension products of ≥ 91 nt were synthesized with a yield of 0.035% of total primer (table S4). Yields of full-length products are limited by a number of factors, including chain termination and ribozyme and product degradation (18). Together, these result in a 7% termination probability (on average) per template position (table S4).

Sequence generality. tC19 enabled the synthesis of some long RNAs, but its polymerase activity, like that of its parent R18, remained template-dependent. Although capable of long primer extension on favorable templates, synthesis from a majority of RNA template sequences was limited. To improve generality, we performed a second series of selection experiments starting from a library of 5×10^7 randomly mutated R18 genes. This starting pool was subjected to three rounds of low-stringency CBT selection to deplete it of detrimental mutations. We then generated new combinations of neutral and beneficial mutations through recombination (19), before applying five additional rounds of increasingly stringent CBT selection, using two distinct selection templates to encourage the emergence of generality (table S3), after which the activity of this polyclonal pool rose to exceed wild-type R18 activity. Screening by RPA identified a clade

of ribozymes (fig. S4c) with substantially improved activity comprising between three and five mutations. We analyzed the contributions of these mutations to disentangle their effects on RNA polymerase activity (fig. S9). Combination of the most favorable mutations (C60U, G93A, G95A, and A159C) in a single RNA molecule yielded Z (Fig. 1D), a ribozyme RNA polymerase with improved sequence generality (fig. S10). Combining Z's core mutations with the 5' extension of tC19 yielded the hybrid ribozyme tC19Z (Fig. 1E) with improved sequence generality and polymerase activity. Although still not independent of the template sequence, this ribozyme outperformed all of its parent ribozymes (R18, tC19, and Z), synthesizing longer extension products on a range of different primer-template sequences (designed to comprise approximately equal representation of all four nucleobases, as well as all 16 dinucleotide combinations) (Fig. 4A). tC19Z also exhibited a further improvement in fidelity (8.8×10^{-3}), as determined by sequencing of full-length extension products (fig. S8).

Hammerhead ribozyme synthesis by tC19Z.

The improvements in RNA polymerase activity and fidelity in the tC19Z ribozyme encouraged us to explore the synthesis of an RNA sequence that encodes a catalytic activity: a hammerhead nuclease ribozyme. To facilitate the synthesis of sufficient amounts of full-length ribozyme for characterization, we chose a minimal version of the hammerhead endonuclease designed for therapeutic applications (20) (Fig. 4B). In contrast to R18, the tC19Z RNA polymerase ribozyme could synthesize full-length (≥ 24 nt) hammerhead minizymes (Fig. 4C, left, and fig. S11). Both the +24-nt minizyme and a pool of longer extension products (≥ 27 nt) exhibited catalytic activity and performed sequence-specific cleavage of a cognate substrate RNA (Fig. 4C, right).

The tC19Z phenotype arises from the contributions of four mutations (C60U, G93A, G95A,

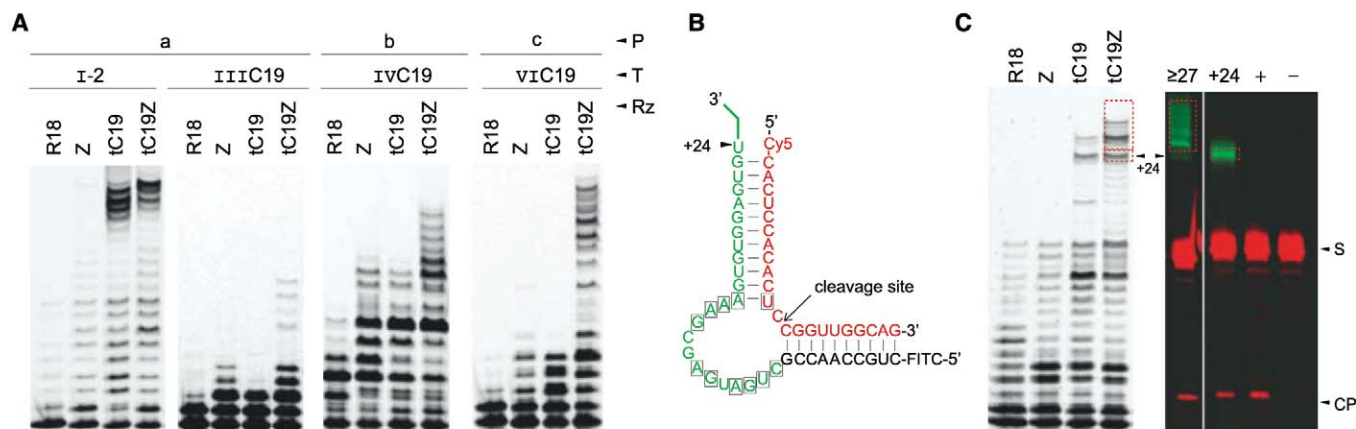
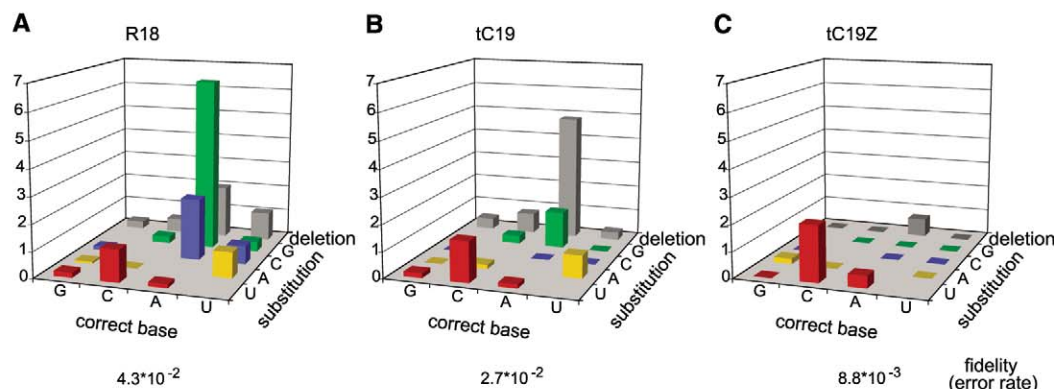


Fig. 4. Generality and hammerhead synthesis. (A) Primer extension by R18, Z, tC19, and tC19Z on different primer-template duplexes (primer, P; template, T; ribozyme, Rz). (B) Secondary structure of hammerhead endonuclease minizyme with ribozyme-synthesized segment (green) and substrate (red). Essential catalytic residues are boxed. (C) Fluorescent primer extension on minizyme templates by ribozymes R18, Z, tC19, and tC19Z

(left). tC19Z-synthesized extension products long enough to form a symmetrical minizyme with the substrate (+24 and ≥ 27 , red boxes) were prepared (fig. S11) and tested for endonuclease activity (right). One to 3% of substrate is cleaved by the control minizyme (+) and by both tC19Z-synthesized minizymes (+24 and ≥ 27) with comparable efficiencies (table S5), but not in their absence (–) (substrate, S; cleavage product, CP).

Fig. 5. RNA extension fidelity. Error spectra (defined, on the y axes, as the average percentage of incorrect nucleotides inserted, per position) of full-length extension products synthesized by RNA polymerase ribozymes R18 (A), tC19 (B), and tC19Z (C). The error rate denotes the average probability of a mutation per nucleotide position (fig. S8). Fidelity data for R18 is derived from Attwater *et al.* (18).



and A159C), as well as a short 5'-extension (5'-GUCAUUGAAAA) to the parental R18 ribozyme. The C60U mutation incrementally enhances RNA polymerase activity; it is the only mutation selected in the catalytic core and changes a G-C base pair to a weaker G-U wobble pair in the central P6 stem. The G93A and G95A mutations in the P2 region disrupt interaction with (and render ribozyme activity independent of) the stem oligonucleotide. G93A and G95A may be necessary to tolerate a single-stranded P2 region in the absence of stem or, alternatively, could promote new interactions with the primer-template duplex or the ribozyme processivity domain. A159C is located in the processivity domain (residues 98 to 187), as part of a large asymmetric internal loop. A159C allows the formation of a new G133:C159 base pair, which augments a potential 4-base pair stem (P12) formed by bulge segments A129 to U132 (5'-ACCU) and A160 to U163 (5'-AGGU) (Fig. 1, D and E). Indeed, G133U, a separate beneficial mutation that was isolated in the selection, would stabilize the P12 stem in an identical way, by promoting formation of an U133:A159 base pair. These two mutations, although beneficial individually [with A159C superior to G133U (fig. S9)], negate one another's effects when combined. This strongly suggests that the selected trait is indeed the formation of a new base pair between positions 133:159, presumably to reshape the structure of the processivity domain. Indeed, a recent study on the processivity domain of the closely related B6.61 RNA polymerase ribozyme also suggested the presence of a 4-base pair P12 stem (21).

The ssC₁₉ sequence tag emerged rapidly in the selection and appears to mediate interaction with the 5' end of the template strand. This interaction is reminiscent of the recognition of target mRNAs by the prokaryotic ribosome through the Shine-Dalgarno sequence (22) and primordial mechanisms of template recognition as proposed by the genomic tag hypothesis (23). Such recognition might have been advantageous in a prebiotic setting, where an RNA polymerase ribozyme would not have evolved in isolation but in the presence of a large number of unrelated RNA oligomers. In such an environment, specific interactions between a replicase and cognate templates via a recognition tag that promotes

polymerization (Fig. 2C) could have supported a primitive form of kin selection, which would enable self-replication and Darwinian evolution even in the absence of compartmentalization in protocellular entities.

RNA replication must proceed with a minimum fidelity, as defined by the "error threshold" (24, 25), to avoid corruption of encoded genetic information. tC19Z contains the C60U mutation in the catalytic core, as well as several other mutations that could potentially affect its fidelity. We determined aggregate fidelities (both deletion mutations and nucleotide misincorporations) of the different ribozymes by sequencing full-length extension products (Fig. 5). This method measures the fidelity of the ribozyme for the synthesis of a given sequence (7, 18), however, truncated extension products are not probed for potential errors (see supporting online material text S1). By this measure, we determined tC19Z's fidelity as 8.8×10^{-3} (fig. S8), which suggested an improvement compared with both the parent R18 (4.3×10^{-2}) (18) and the intermediate tC19 (2.7×10^{-2}) ribozymes. Some RNA sequences are synthesized by tC19Z with even greater accuracy. Analysis of the hammerhead ribozymes synthesized by tC19Z revealed just two mutations in 37 sequenced clones ($2 \times \text{G} \rightarrow \text{A}$ transitions in 999 nt), which indicates a fidelity of 2×10^{-3} —a 20-fold improvement over the fidelity of the parent R18 ribozyme. Although comparisons are imperfect because of some differences between the sequences sampled for the different ribozymes, there is a notable reduction in A $\rightarrow$ G transition mutations in the mutation spectrum of tC19Z (and to a lesser extent in tC19), which suggests a reduced susceptibility to misincorporation of G opposite template U. On the other hand, the reverse C $\rightarrow$ U transition mutation (denoting misincorporation of U opposite template G) is not decreased, indicating an asymmetric, i.e., strand-specific recognition of the G•U wobble pair by tC19Z.

The R18 RNA polymerase ribozyme had been widely considered to be an evolutionary dead-end, trapped in a local activity optimum and largely refractory to further substantial evolution (26). Starting from R18, we have shown that the CBT selection method, in combination with RNA engineering, can yield polymerase ribozymes, such as

tC19Z, that display enhanced polymerase activity and fidelity, as well as generality, and allow the ribozyme-catalyzed transcription of an active ribozyme from an RNA template.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S11

Tables S1 to S5

References

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Ensemble Asteroseismology of Solar-Type Stars with the NASA Kepler Mission

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In addition to its search for extrasolar planets, the NASA Kepler mission provides exquisite data on stellar oscillations. We report the detections of oscillations in 500 solar-type stars in the Kepler field of view, an ensemble that is large enough to allow statistical studies of intrinsic stellar properties (such as mass, radius, and age) and to test theories of stellar evolution. We find that the distribution of observed masses of these stars shows intriguing differences to predictions from models of synthetic stellar populations in the Galaxy.

An understanding of stars is of central importance to astrophysics. Uncertainties in stellar physics have a direct impact on fixing the ages of the oldest stellar populations (which place tight constraints on cosmologies) as well as on tracing the chemical evolution of galaxies. Stellar astrophysics also plays a crucial role in the current endeavors to detect habitable planets around other stars (1–5). Accurate data on the host stars are required to determine the sizes of planets discovered by the transit method, to fix the locations of habitable zones around the stars, and to estimate the ages and to understand the dynamical histories of these stellar systems. Measurements of the levels of stellar activity and their variations over time (6) provide insights into planetary habitability, the completeness of the survey for extrasolar planets, and the surface variability shown by our own Sun, which has very recently been in a quiescent state that is unique in the modern satellite era (7, 8).

New insights are being made possible by asteroseismology, the study of stars by observations of their natural, resonant oscillations (9, 10). Stellar oscillations are the visible manifestations of standing waves in the stellar interiors. Main-sequence and subgiant stars whose outer layers are unstable to convection (solar-type stars) display solarlike oscillations that are predominantly acoustic in nature, excited by turbulence in the convective envelopes (11, 12). The dominant oscillation periods are minutes in length and give rise to variations in stellar brightness at levels of typically just a few parts per million. The frequencies of the oscillations depend on the internal structures of the stars, and their rich information content means

that the fundamental stellar properties (e.g., mass, radius, and age) can be determined to levels that are difficult to achieve by other means and that the internal structure and dynamics can be investigated in a unique way.

Helioseismology has provided us with an extremely detailed picture of the internal structure and dynamics of the Sun, including tests of basic physics (13–15). Such investigations are beginning to be possible for other stars. Over the past decade, the quality of seismic observations on other solar-type stars has been improving steadily, from ground-based spectroscopy (16–18) and the French-led CoRoT (Convection Rotation and Planetary Transits) satellite (19, 20). Now, Kepler is providing ultraprecise observations of variations in stellar brightness (photometry), which are suitable for the study of solarlike oscillations (21). During the first 7 months of science operations, more than 2000 stars were selected for observation for 1 month each with a cadence rapid enough to perform an asteroseismic survey of the solar-type population in the Kepler field of view. Here, we report the detection of solarlike oscillations in 500 of those stars. Previously, this type of oscillation had been detected in only about 25 stars.

As is evident from the frequency spectra of the oscillations exhibited by nine stars from the ensemble (Fig. 1), solarlike oscillators present a rich, near-regular pattern of peaks that are the signatures of high-order overtones. The dominant frequency spacing is the so-called large separation, $\Delta\nu$, between consecutive overtones (22). The average large separation scales approximately with the square root of the mean density of the

star. The observed power in the oscillations is modulated in frequency by a Gaussian-like envelope. The frequency of maximum oscillation power, ν_{max} , scales approximately as $gT_{\text{eff}}^{-1/2}$, where $g \propto M/R^2$ is the surface gravity and T_{eff} is the effective temperature of the star (23, 24).

Figure 2 shows all the stars on a conventional Hertzsprung-Russell diagram, which plots the luminosities of stars against T_{eff} . The temperatures were estimated (25) from multicolor photometry

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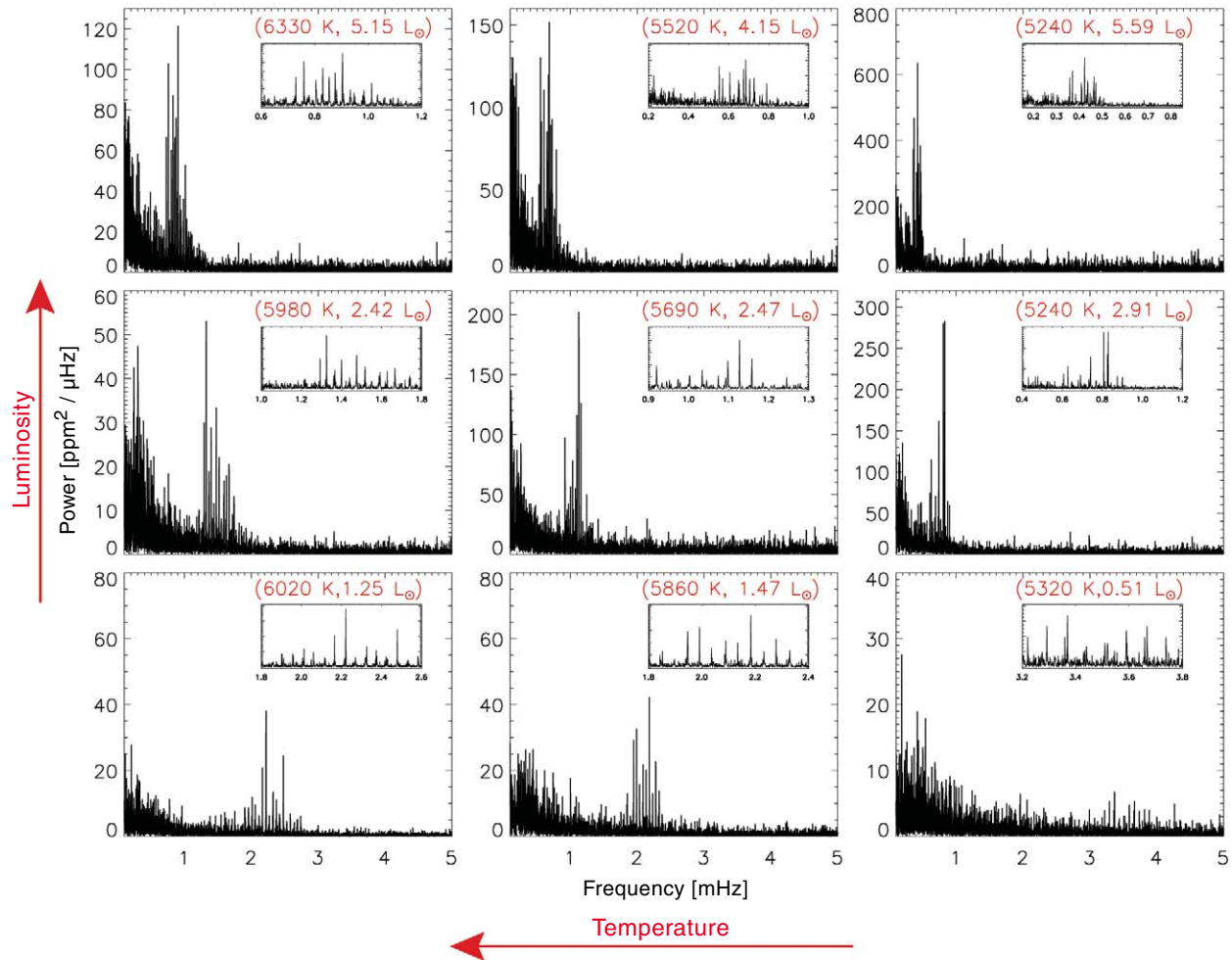


Fig. 1. Frequency spectra of the oscillations exhibited by nine stars from the ensemble. Each spectrum shows a prominent Gaussian-shaped excess of power because of the oscillations, centered on the frequency $\nu_{\max}$. (**Insets**) Clearer views of the near-regular spacings in frequency between individual modes of

oscillation within each spectrum. The stars are arranged by intrinsic brightness [in units of solar luminosity ($L_{\odot}$)] and temperature, with intrinsically fainter stars showing weaker, less prominent oscillations than their intrinsically brighter cousins. ppm, parts per million.

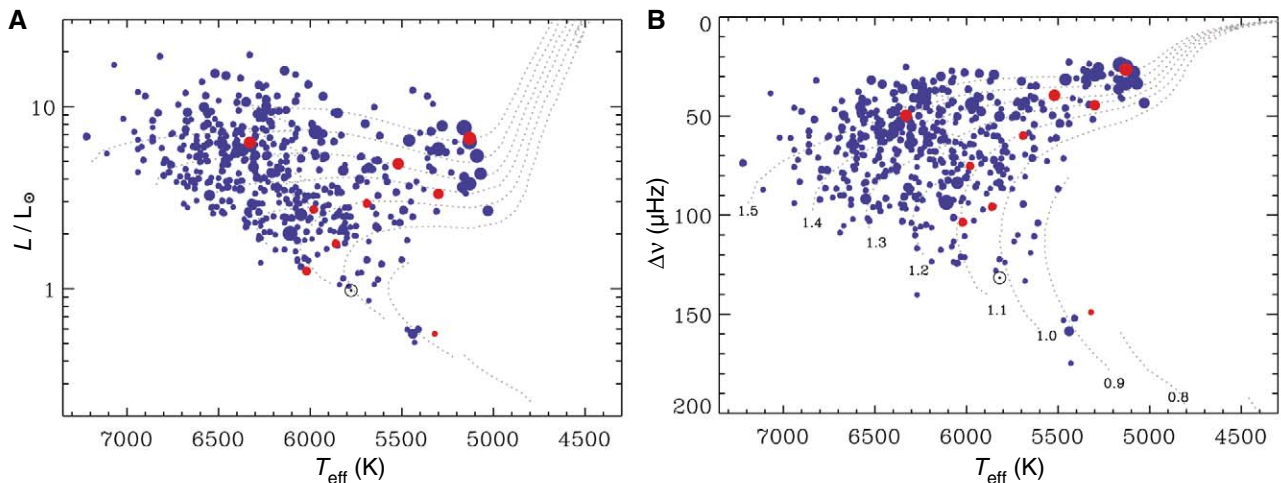
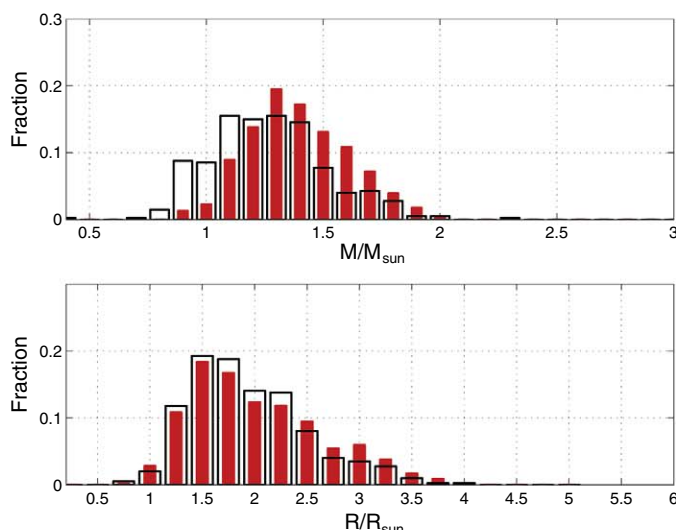


Fig. 2. (A) Estimates of the luminosities of the stars (in units of the solar luminosity) of the ensemble of Kepler stars showing detected solarlike oscillations, plotted as a function of effective temperature. Stars from Fig. 1 are plotted with red symbols. (B) Average large frequency separations, $\Delta\nu$, against effective temperature. The symbol sizes are directly proportional to the prominence of the detected oscillations (i.e., the

signal-to-noise ratios). These ratios depend both on stellar properties (e.g., the photometric amplitudes shown by the oscillations and the intrinsic stellar backgrounds from convection) and the apparent brightness of the stars. The dotted lines show predicted evolutionary tracks (33) for models of different stellar mass (0.8 to 1.5 solar masses, in steps of 0.1). The Sun is marked with a solar symbol ($\odot$).

Fig. 3. Black lines show histograms of the observed distribution of masses (**top**) and radii (**bottom**) of the Kepler ensemble (27). In red, the predicted distributions from population synthesis modeling, after correction for the effects of detection bias (27). The population modeling was performed by using the TRILEGAL code (34, 35).



available in the Kepler Input Catalog (26). Luminosities were estimated from the temperatures and the seismically estimated radii [see below and (27)]. We also plot $\Delta\nu$ against temperature, and, just like the conventional diagram, this asteroseismic version delineates different types of stars and different evolutionary states (the $v_{\max}$ version is similar). Main-sequence stars, burning hydrogen into helium in their cores, lie in a diagonal swathe (from the lower right to top left) on each diagram. Both asteroseismic parameters, $\Delta\nu$ and $v_{\max}$, decrease along the main sequence toward hotter solar-type stars, where surface gravities and mean densities are lower than in cooler stars (and luminosities are higher). After exhaustion of the core hydrogen, stars eventually follow nearly horizontal paths in the luminosity plot toward lower temperatures as they evolve as subgiants, before turning sharply upward to become red giants (28, 29). The values of $\Delta\nu$ and $v_{\max}$ decrease comparatively rapidly through the subgiant phase. Detailed information on the physics of the interiors of these stars is emerging from analysis of Kepler data (30).

We have detected solarlike oscillations in relatively few stars that have $\Delta\nu$ and $v_{\max}$ larger than the solar values. These stars are intrinsically fainter and less massive than the Sun, and we see fewer detections because the intrinsic oscillation amplitudes are lower than in the hotter main-sequence and evolved subgiant stars. This detection bias means that the most populous cohort in the ensemble is that comprising subgiants. Subgiants have more complicated oscillation spectra than main-sequence stars. The details of the spectra depend on how, for example, various elements are mixed both within and between different layers inside the stars. Seismic analysis of the Sun has already shown that merely reproducing the luminosity and temperature of a star will not guarantee that the internal structure, and hence the underlying physics, is correct. This inspired the inclusion of additional physics, such as the settling over time of chemical elements because

of gravity, in stellar models (13). The Sun is a relatively simple star compared with some of the solar-type stars observed by Kepler.

We made use of the $\Delta\nu$ and $v_{\max}$ of the ensemble together with photometric estimates of the temperatures to estimate the masses and radii of the stars in a way that is independent of stellar evolutionary models—by using the so-called direct-method of estimation (27)—and then compared the observed distributions with those predicted from synthetic stellar populations (Fig. 3). The synthetic populations were calculated by modeling the formation and evolution of stars in the Kepler field of view, which lies in the Cygnus region of the Orion arm of our Galaxy, the Milky Way (27). This modeling requires descriptions of, for example, the star-formation history (including the frequency of occurrence of stars with various masses), the spatial density of stars in the disc of the Milky Way, and the rate at which the Galaxy is chemically enriched by stellar evolution (31).

Previous population studies have been hampered by not having robust mass estimates on individual stars (31). Precise estimates of masses of solar-type stars had been limited principally to stars in eclipsing binaries (32). The Kepler estimates add substantially to this total and in numbers that are large enough to do statistical population tests by using direct mass estimates, which had not been possible before.

Whereas the distributions of stellar radii in Fig. 3 are similar, the same cannot be said for the mass distributions. We have quantified the significance of the differences by using statistical tests. Differences in radius were judged to be marginally significant at best. In contrast, those in mass were found to be highly significant (>99.99%) (27). The observed distribution of masses is wider at its peak than the modeled distribution and is offset toward slightly lower masses.

Tests suggest that, for the bulk of the stars, bias in the estimated masses and radii is no

larger than the estimated uncertainties (27). On the assumption that the observed masses and radii are robust, this result may have implications for both the star-formation rate and the initial mass function of stars. Mixing or overshooting of material between different layers (including stellar cores) and the choice of the so-called mixing length parameter, which measures the typical length scale of the convection and is one of the few free parameters in stellar evolution theory, may also be relevant. It is yet to be tested whether the expected small fraction of unresolved binaries could have contributed to the mass discrepancy.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6026/213/DC1

Materials and Methods

Figs. S1 to S3

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HD 181068: A Red Giant in a Triply Eclipsing Compact Hierarchical Triple System

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Hierarchical triple systems comprise a close binary and a more distant component. They are important for testing theories of star formation and of stellar evolution in the presence of nearby companions. We obtained 218 days of Kepler photometry of HD 181068 (magnitude of 7.1), supplemented by ground-based spectroscopy and interferometry, which show it to be a hierarchical triple with two types of mutual eclipses. The primary is a red giant that is in a 45-day orbit with a pair of red dwarfs in a close 0.9-day orbit. The red giant shows evidence for tidally induced oscillations that are driven by the orbital motion of the close pair. HD 181068 is an ideal target for studies of dynamical evolution and testing tidal friction theories in hierarchical triple systems.

The Kepler space mission is designed to observe continuously more than 10^5 stars, with the ultimate goal of detecting a sizeable sample of Earth-like planets around main-sequence stars (*1*). We obtained 218 days of Kepler photometry (2–4) of HD 181068 [also Kepler Input Catalog (KIC) 5952403], a star with magnitude $V = 7.1$ and a distance of about 250 pc. It had been previously identified as a single-lined spectroscopic binary (*5*), but there have been no reports of eclipses.

The data were obtained in long-cadence (LC) mode (one point every 29.4 min) over 218 days using quarters 1, 2, and 3. Our observations reveal a very distinctive light curve. It shows eclipses every ~ 22.7 days and slow variations in the upper envelope (Fig. 1A) that are likely caused by ellipsoidal distortion of the primary component. There are also very regular and much narrower eclipses (Fig. 1B). These minima have alternating depths, corresponding to a close pair (B and C), with an orbital period of ~ 0.9 day. The 22.7-day eclipses all have similar depths, but there are subtle differences between consecutive minima. Radial velocity observations [section 1.2 of (6)] show that the true orbital period of the BC pair around the A component is 45.5 days. The narrow 0.9-day eclipses essentially disappear during both types of the deep minima, implying that the three stars have very similar surface bright-

nesses, so that when the BC pair is in front of A, their mutual eclipses do not change the total amount of light coming from the system (in accordance with the nearly equal depths of the two deep minima). When the BC pair is in front of A, the BC's secondary eclipses appear as tiny brightenings (Fig. 1, D and F), showing that the surface brightness of B is almost equal to that of A, whereas C is a bit fainter, so that its disappearance behind B allows the extra light from A to reach us.

The observed changes in the eclipses of the BC pair and the radial velocity variations of the A component confirm that the A and BC systems are physically associated and not a chance alignment. Their periods are $P_{BC} = 0.90567(2)$ day and $P_{A-BC} = 45.5178(20)$ days (numbers in parentheses refer to the standard error in the last digits). Given the shallow depths of the eclipses, star A must be the most luminous object in the system. In addition to the eclipses, there are brightness fluctuations during the long-period minima that imply that component A is also an intrinsic variable star with a mean cycle length close to half the shorter orbital period, possibly indicating tidally induced oscillations.

In addition, there were several flarelike events in the light curve that usually lasted about 6 to 8 hours. We checked the Kepler Data Release Notes (7) for documented instrumental effects in the vicinity of the “flares” but found none. More-

over, almost all flares appear right after the shallower minimum of the BC pair, suggesting that this activity might be related to the close pair.

We looked for optically resolved companion(s) with a 1-m telescope [section 1.1 of (6)] but found none. We also obtained 38 high-resolution optical spectra to measure the orbital reflex motion of the A component (6) (fig. S1). The orbital parameters for the wider system (Table 1) reveal that star A revolves on a circular orbit, which has an orbital period twice the separation of the two consecutive flat-bottomed minima in the light curve (6). Long-baseline interferometry using the PAVO (Precision Astronomical Visible Observations) beam combiner (8) at the CHARA [Center for High Angular Resolution Astronomy (9)] Array show that the angular diameter of HD

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Fig. 1. Kepler-band light curves of HD 181068 from observations in LC mode: **(A)** the full 218 days and **(B)** a 28-day segment showing two consecutive deep minima. Time is expressed in barycentric Julian date. **(C–F)** Close-ups of two secondary minima and two primary minima of the 45.5-day eclipses. The dashed and dotted lines mark the primary and the secondary minima of the 0.9-day eclipses, respectively. The discontinuities in **(A)** correspond to the telescope rolls at the end of each quarter.

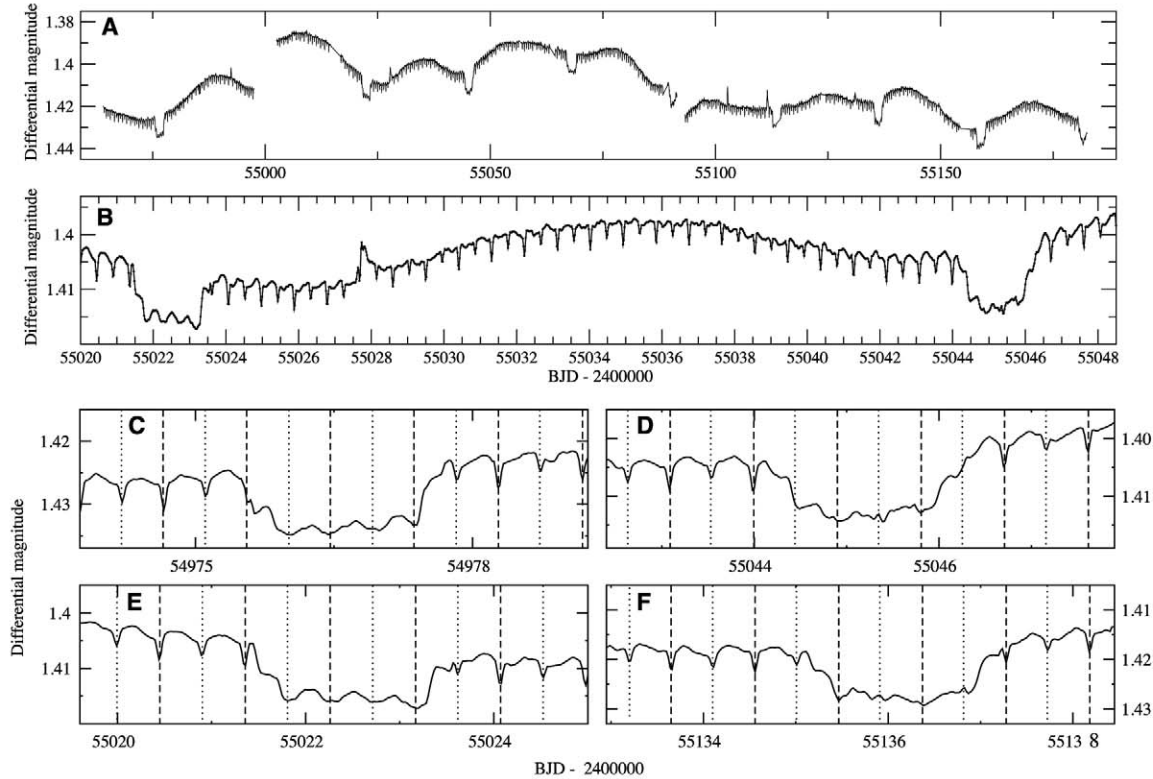


Table 1. Orbital elements for the wider system derived from the A component's radial velocity curve (fig. S1). K_2 , the velocity amplitude of component A; v_γ , the center-of-mass velocity of the system; e_2 , the eccentricity of the wide pair; $f(m)$, the mass function.

Element	Value
P_{A-BC}	45.5178 days (fixed)
$T_{\text{Min } 1}$	$2,455,454.573 \pm 0.095$ (fixed)
K_2	$37.195 \pm 0.053 \text{ km s}^{-1}$
v_γ	$6.993 \pm 0.011 \text{ km s}^{-1}$
e_2	0.0 (fixed)
$f(m)$	$0.24 \pm 0.02 M_\odot$

181068 A, corrected for limb darkening (10), is $\theta_{\text{LD}} = 0.461 \pm 0.011$ milli-arc sec (mas) (Fig. 2) [see (6) for details].

Combining the measured angular diameter with the Hipparcos parallax of 4.0 ± 0.4 mas (11), we find the linear radius of the primary component to be $R = 12.4 \pm 1.3 R_\odot$ (where $R_\odot$ is the radius of the Sun). With use of the spectroscopically determined $T_{\text{eff}} = 5100 \pm 200$ K, this implies a luminosity of $L = 93 \pm 19 L_\odot$ (where $L_\odot$ is the luminosity of the Sun). This value matches that found from the Hipparcos parallax and the apparent magnitude. We also estimated the absolute magnitude of HD 181068 A on the basis of the Wilson-Bappu effect (12), which correlates the width of the chromospheric Ca II K emission line at 3934 Å with the V -band absolute magnitude. With the latest calibration (13), the measured width of the emission core, $W_0 = 72.8 \text{ km s}^{-1}$ (6), implies an absolute brightness of $M_V = -0.3$ mag-

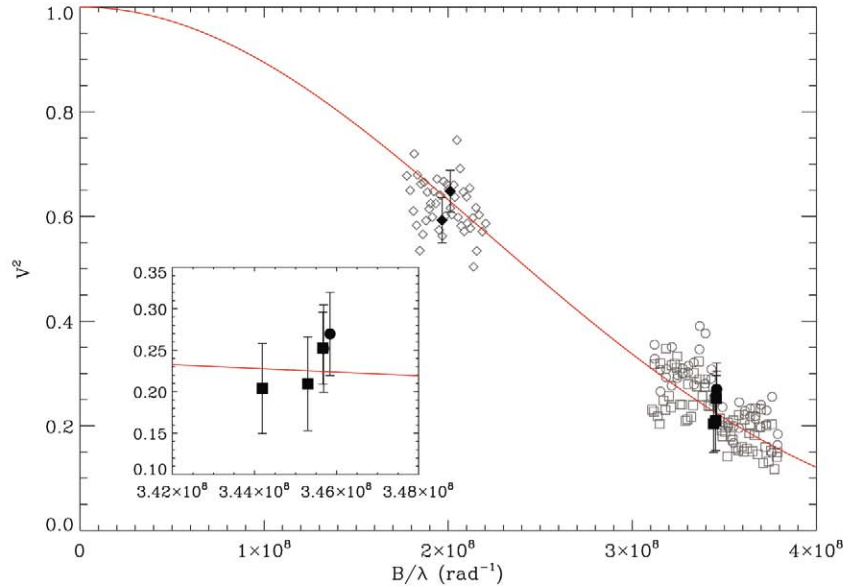


Fig. 2. Squared visibility versus spatial frequency (projected baseline length over wavelength) from PAVO on CHARA. Gray points show all collected measurements; black symbols show the average of each scan over all wavelength channels; the error bars show the standard error of the mean. Each symbol type corresponds to a different night of observations. The solid line is the best-fitting model. **(Inset)** A close-up of the observations at the longer baselines.

nitude, which matches the parallax and the interferometric results.

We estimated the mass of HD 181068 A by comparing the effective temperature and the luminosity with evolutionary tracks from the BASTI database (14). We obtained $M_A \sim 3.0 \pm 0.4 M_\odot$ (where $M_\odot$ is the mass of the Sun), corresponding to a red giant, possibly in the He-core burning

phase of its evolution (15). The full spectral energy distribution constructed by using all published broad-band optical magnitudes and infrared flux values (6) does not show any excess in comparison to a 5200-K photospheric model, indicating that no detectable circumstellar dust, possibly from mass-loss processes on the red giant branch, is present.

We have constrained the parameters of the BC pair by modeling the short-period eclipses in the Kepler band using the jktebop code (16, 17). The ratio of the radii of the B and C components is poorly constrained at present, partly because of the low sampling rate of the Kepler LC data. The A component contributes 99.29% of the system light in the Kepler passband, and the BC pair contribute 0.44% and 0.27%, respectively. Taking the *V*-band absolute magnitude of HD 181068 A to be $M_V(A) = -0.3$ and assuming that our results for the Kepler passband are representative of the *V* band, we find $M_V(B) = 5.6$ and $M_V(C) = 6.1$. Such absolute magnitudes indicate spectral types of G8 V and K1 V for stars B and C, respectively (18). Because we do not have independent measurements of T_{eff} for the BC pair, we can only estimate their masses based on their spectral types. This indicates that their masses are about $0.7 \pm 0.1 M_{\odot}$ each (6).

One puzzling feature of the system is the short-period fluctuations that have the largest amplitudes when the BC pair is behind star A, while remaining apparent with a slowly changing amplitude in all the other phases of the wide orbit. We have investigated this variability of HD 181068 A with a detailed frequency analysis and a comparison to other red giant stars that have similar properties (6). The frequency content of the light curve suggests an intimate link to tidal effects in the triple system, with the first four dominant peaks in the power spectrum identifiable as simple linear combinations of the two orbital frequencies. On the other hand, solarlike oscillations (meaning those excited by near-surface convection, as in the Sun but also observed in red giants) that are expected to produce an equidistant series of peaks in the power spectrum are not visible, even though all stars with similar parameters in the Kepler database do show clear evidence of these oscillations. In other words, the convectively driven solarlike oscillations that we would expect to see in a giant of this type seem to have been suppressed (6).

In a recent compilation of 724 triple stars (19), there is only one system with an outer orbital period shorter than that of HD 181068 (λ Tau, for which $P_{\text{out}} = 33.03$ days). Carter *et al.* (20) reported the discovery of KOI-126 with a similarly short outer period ($P_{\text{out}} = 33.92$ days). Extremely compact hierarchical triple systems form a very small minority of hierarchical triplets, with only 7 of the catalogized 724 systems having outer periods shorter than 150 days. Furthermore, HD 181068 and KOI-126 have the highest outer mass ratios [~ 2.1 and 3, defined as $m_A/(m_B + m_C)$] among the known systems. In 97% of the known hierarchical triplets before the Kepler era, the mass of the close binary exceeded that of the wider companion, and even the largest outer mass ratio remained under 1.5. It is not yet clear whether this rarity of such systems is caused by an observational selection effect or has an underlying stellar evolutionary or dynamical explanation.

Its properties make HD 181068 an ideal target for dynamical evolutionary studies and for testing tidal friction theories. Because of its compactness and its massive primary, we can expect short-term orbital element variations on two different time scales of 46 days (i.e., with period of P_{A-BC}) and about 6 years (P_{A-BC}^2/P_{BC}), the time scale of the classical apsidal motion and nodal regression (21), which for the triply eclipsing nature could be observed relatively easily.

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Supporting Online Material

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Figs. S1 to S3

Tables S1 to S3

References

SOM Data

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Surface-Plasmon Holography with White-Light Illumination

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The recently emerging three-dimensional (3D) displays in the electronic shops imitate depth illusion by overlapping two parallax 2D images through either polarized glasses that viewers are required to wear or lenticular lenses fixed directly on the display. Holography, on the other hand, provides real 3D imaging, although usually limiting colors to monochrome. The so-called rainbow holograms—mounted, for example, on credit cards—are also produced from parallax images that change color with viewing angle. We report on a holographic technique based on surface plasmons that can reconstruct true 3D color images, where the colors are reconstructed by satisfying resonance conditions of surface plasmon polaritons for individual wavelengths. Such real 3D color images can be viewed from any angle, just like the original object.

Noble metal films, such as silver and gold foil, contain free electrons that collectively oscillate and propagate as the surface

wave in optical frequency region. The quantum of this surface wave is called surface plasmon polariton (SPP) (1). The electromagnetic field gen-

erated by SPP can be enhanced and strongly confined spatially in the near field (with the distance less than the wavelength) from the metal surface as a nonirradiative evanescent field (2, 3). The ability to confine and enhance the optical field to the vicinity of the metal surface or nanometal particle has been applied to immuno-sensor (4), fluorescence sensor (5), solar cell (6), plasmonic laser (7, 8), nanomicroscopy (9, 10), super-lens (11, 12) and photodynamic cancer cell treatment (13).

We report an application of SPP to three-dimensional (3D) color holography with white-

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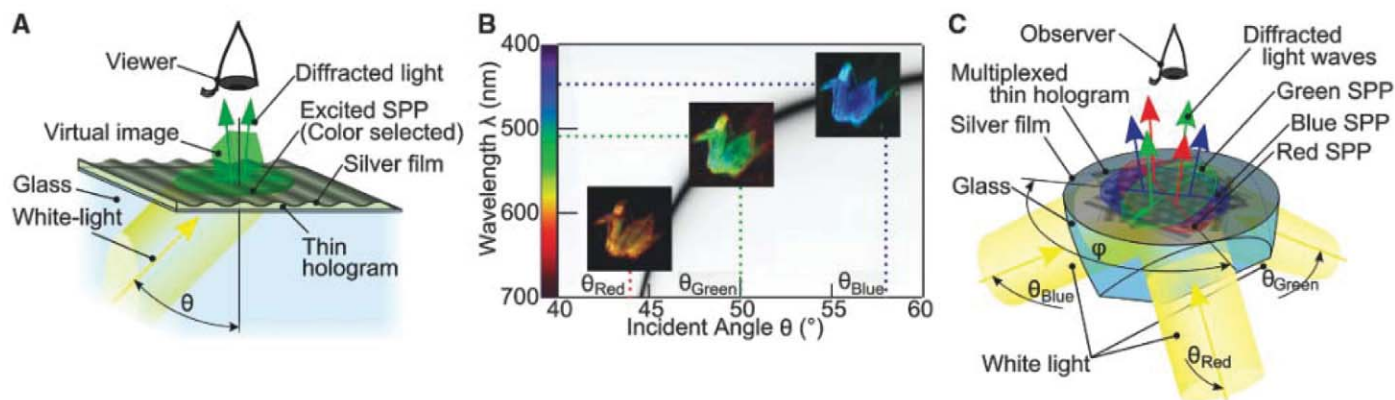


Fig. 1. Surface plasmon hologram and its color reconstruction with white-light illumination. **(A)** The SPP hologram is illuminated by white light at a given angle θ in high-index medium. Surface plasmons of a selected color are excited and diffracted by the SPP hologram to reconstruct the wavefront of the object. **(B)** Dispersion curve of the SPP hologram in reconstruction as a function of the incident

angle of white light. The 3D images of red, green, and blue cranes made of paper are obtained at different angles with white-light illumination. This curve was obtained through calculations based on Fresnel's equations. **(C)** Reconstruction of a color object through SPP hologram. The hologram is illuminated simultaneously with a white light in three directions at different angles θ and ϕ for each.

light illumination. The first idea to use plasmons for holography was published as a reflection type by Cowan (14), and since then authors have reported holographic reconstruction of transmission type (15–17). In those configurations, plasmons have been used for enhancing the diffraction efficiency. In this report, we use color selectivity of SPPs for holographic color reconstruction with white-light illumination.

We recorded the hologram on a photoresist as an interference pattern between a light field coming from the object as scattered light and the unscattered reference beam. Exposure was repeated three times with rotation of the illumination angle for a single color hologram recording. Instead of rotation, one can also use three lasers simultaneously to obtain the hologram in a single exposure. A thin metal film is then coated on the photoresist hologram, which is precoated on a glass plate. For image reconstruction, the SPP is excited by a color component of white light that is incident on the metal film through a prism with an angle satisfying the condition of total internal reflection (Fig. 1A). The SPP associates with a nonradiative evanescent light wave on metal film and then is converted by the grating component of hologram into a radiative light field, which represents the reconstructed wavefront of light that scattered at the object. The reconstruction of the prerecorded object is seen with the eyes through an SPP hologram in color. The reference beam or the zeroth-order diffraction as background beam does not exist in reconstruction for this configuration because the illumination of the hologram is made by total internal reflection.

Figure 1B shows the dispersion curve of SPP with wavelength λ as a function of the incident angle θ of illumination (excitation). This relationship is given as

$$\theta = \sin^{-1} \left(\frac{1}{n_{\text{glass}}} \sqrt{\frac{n_m^2 n(\lambda)^2}{n_m^2 + n(\lambda)^2}} \right) \quad (1)$$

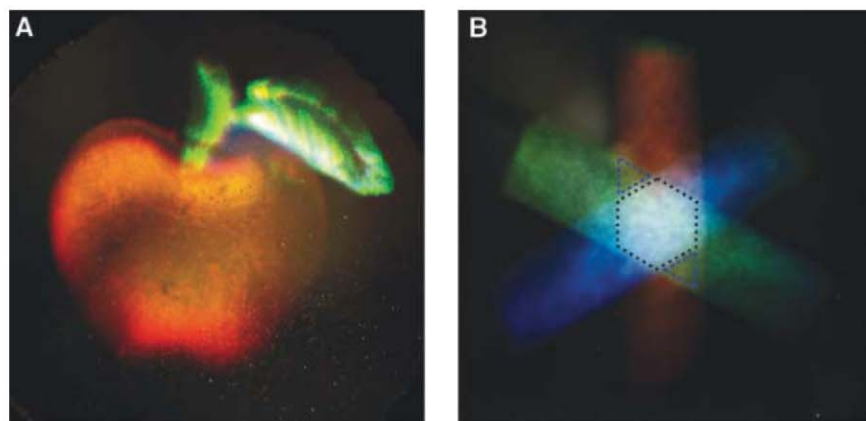


Fig. 2. Reconstruction of three-dimensional color objects through surface plasmon holograms. **(A)** Red apple with green leaf in three dimensions (see movie S1). **(B)** Color bar recorded three times with red, green, and blue by rotating the bar by 120° for each color. In the center, where the three colors overlap, a hexagonal area is reconstructed as white, whereas yellow triangles are reconstructed where red and green overlap.

where n_{glass} , n_m , and $n(\lambda)$ are the refractive index of the glass substrate, effective index of the medium on the metal surface, and the index of metal (which is a function of λ), respectively (2).

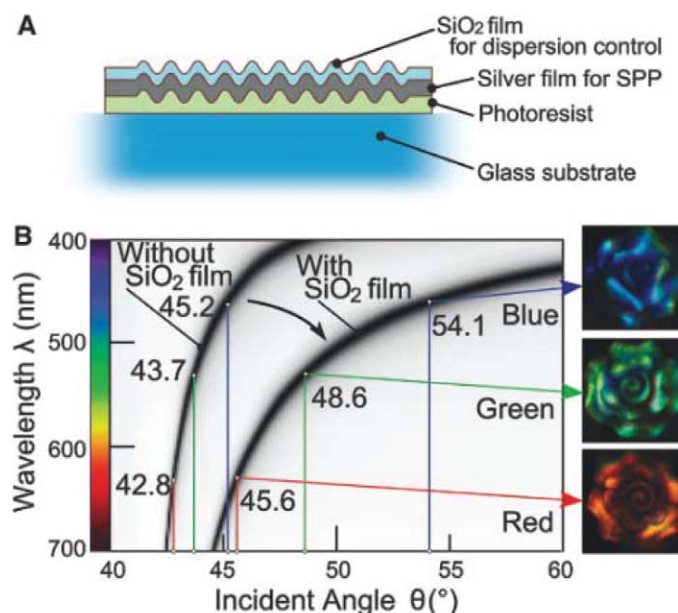
Individual colors are reconstructed by illumination at corresponding incident angles by satisfying the above relationship for each color. Figure 1C shows the optical setup for reconstructing a three-color object in different azimuthal and incident angle sets $[(\phi_R, \theta_R), (\phi_G, \theta_G), \text{ and } (\phi_B, \theta_B)]$ with white-light illumination.

Reconstruction of an object, an apple with a leaf, is seen in 3D from the thin film plasmon hologram (Fig. 2A) (18). A movie of the object taken with a camera moving around the object is provided (movie S1) in the supporting online material. For plasmon color holography, the adjustment of the white color balance is important for representing the natural color of an object or a scene. We obtained the white color balance by carefully controlling the power of the laser with

multiple exposures for red (R), green (G), and blue (B). Figure 2B shows an image of a bar taken by three-time exposure in R, G, and B; each exposure was made by rotating the object (bar) and by taking shots at every 120° of rotation. It includes a white hexagon in the center where all three colors overlap and yellow triangles where red and green bars overlap. For reconstruction of the object, a 100-W Halogen lamp is used.

Figure 3A shows the multilayer system of an SPP hologram. The role of the top-layer dielectric film is to enhance the spectral separation in reconstruction. If this film is absent, the angular separation for the three colors R, G, and B in reconstruction is not large enough (less than 3° between red and blue; more precisely, the angles for the three colors are at 42.8° , 43.7° , and 45.2° , respectively) in a dispersion relationship (Fig. 3B) and makes it difficult to practically reconstruct a color object. If the SiO_2 film is coated, the angular separation between red and blue be-

Fig. 3. Multilayer system and the color dispersion of an SPP hologram. **(A)** Hologram configuration. From the top, dielectric layer (25-nm-thick SiO_2), metal layer (55-nm-thick silver), and dielectric substrate with 25-nm depth modulation (150-nm-thick photoresist on the glass). **(B)** The top SiO_2 layer works for expanding the color dispersion to incident angle. Without the SiO_2 layer on the hologram, the angular separation for color reconstruction is small. Rose pendants of red, green, and blue are separated in the reconstruction, as shown in the insets.



comes as large as 10° due to the higher index n_m of film; more precisely, the angles for the three colors are at 45.6° , 48.6° , and 54.1° , respectively. In Fig. 3B, theoretical curves are plotted from the calculation of a multilayer system based on Fresnel's equations for noncoated and coated SPP holograms. A rose pendant in the figure is decomposed into three colors in reconstruction by choosing the angle for white-light illumination (Fig. 3B).

Our results show that plasmon color holography provides a view of an object or a scene seen naturally and vitally with white-light illumination. A typical amplitude modulation in plasmon hologram is ~ 25 nm (fig. S2), which is much

thinner compared with Lippmann-Denisyuk's color hologram (19) based on Bragg diffraction in volume. The rainbow holograms mounted, for example, on credit cards (20) also reconstruct with white light, where color varies with viewing angle but not with the color distribution in the object. Plasmon holography is advantageous in terms of background-beam-free reconstruction because the illumination light is totally reflected back at the hologram (21). Plasmon holography does not suffer from the ghost produced by the diffraction of ambient light or higher orders of diffraction, because those components are not coupled with SPPs.

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22. S. Kawata conceived the research and planned the experiments. M. Ozaki conducted the experiment and calculations. All authors discussed the results and contributed in preparing the manuscript. The authors thank R. Furutani for discussion and P. Verma for his review.

Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6026/218/DC1
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Movie S1

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The Hot Summer of 2010: Redrawing the Temperature Record Map of Europe

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The summer of 2010 was exceptionally warm in eastern Europe and large parts of Russia. We provide evidence that the anomalous 2010 warmth that caused adverse impacts exceeded the amplitude and spatial extent of the previous hottest summer of 2003. "Mega-heatwaves" such as the 2003 and 2010 events likely broke the 500-year-long seasonal temperature records over approximately 50% of Europe. According to regional multi-model experiments, the probability of a summer experiencing mega-heatwaves will increase by a factor of 5 to 10 within the next 40 years. However, the magnitude of the 2010 event was so extreme that despite this increase, the likelihood of an analog over the same region remains fairly low until the second half of the 21st century.

Increasing greenhouse gas concentrations are expected to amplify the variability of summer temperatures in Europe (1–5). Along with mean warming, enhanced variability results in more frequent, persistent, and intense heatwaves

(6–10). Consistent with these expectations, Europe has experienced devastating heatwaves in recent years. The exceptional summer of 2003 (1, 11–13) caused around 70,000 heat-related deaths, mainly in western and central Europe

(14). In summer 2010, many cities in eastern Europe recorded extremely high values of daytime (for example, Moscow reached 38.2°C), nighttime (Kiev reached 25°C), and daily mean (Helsinki reached 26.1°C) temperatures (fig. S1). Preliminary estimates for Russia referred a death toll of 55,000, an annual crop failure of $\sim 25\%$, more than 1 million ha of burned areas, and $\sim \text{US\$15 billion}$ ($\sim 1\%$ gross domestic product) of total economic loss (15). During the same period, parts of eastern Asia also experienced extremely warm temperatures, and Pakistan was hit by devastating monsoon floods.

In order to characterize the magnitude and spatio-temporal evolution of the 2010 event in a historical context, we used daily mean data sets

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from the two longest reanalyses, which together extend back to 1871 (15–17). The spatial pattern of the maximum summer temperature anomalies (Fig. 1, A to D) reveals that western Russia was in the center of the exceptional warmth at all temporal scales. Weekly to monthly anomalies were particularly pronounced, exceeding the 1970–1999 mean (18) by 10°C—more than 4 standard deviations (SDs). According to reanalyses, other countries (Baltic countries, Belarus, Ukraine, and Kazakhstan) also experienced extreme temperatures that broke the summer records of the last 140 years at many temporal scales [supporting online material (SOM) text].

Figure 1E displays the temporal evolution of areas that were simultaneously affected by record-breaking anomalies for any summer period of 1- to 91-day length. At short time scales (daily to fortnightly), new historical maxima were first observed in late July 2010 and persisted until the second week of August. During that time, extensive fires across western Russia killed 53 people and made 3500 people homeless, and Moscow suffered a devastating rise in mortality, smoke fire, and air pollution (15). The record-breaking pattern is not symmetric in time, indicating that warm conditions had started already in July and ended abruptly by mid-August. Be-

tween mid-July and early August, a record-scale area of more than 2 million km² registered unprecedented anomalies at the 15- to 61-day time scales. These results reflect the extraordinary nature of the 2010 event and confirm that most of the record-breaking values highlighted in Fig. 1, A to D, occurred simultaneously.

The most evident features associated with the 2010 event were (i) quasi-stationary anticyclonic circulation anomalies over western Russia (fig. S5) and (ii) deficit of January-to-July 2010 accumulated precipitation and early spring snow cover disappearance in western-central Russia (fig. S6). High-pressure systems are well-known to produce warm conditions at surface by enhancing subsidence, solar heating, and warm-air advection (19–21). The lack of water availability results in a continuous reduction of soil moisture and enhanced sensible heat fluxes that exacerbate the strength of summer heatwaves (20–22).

Unlike 2010, the summer of 2003 was characterized by two extreme periods (centered on 15 June and the first fortnight of August) (12) that contributed to the rise of the number of locations with temperatures beyond their historical maxima at seasonal time scales. However, the 2010 event exceeded the 2003 episode in terms of amplitude and spatial extent. Thus, the maximum extension of areas experiencing record-breaking temperatures in 2003 was ~1 million km², which is considerably lower than that of 2010. The intensity of the 2003 event was also ~1 to 2 SDs weaker for most of the subseasonal time scales (fig. S7).

Despite the distinctive spatio-temporal evolution of the 2003 and 2010 events, their record-breaking anomalies reached comparable amplitude and extension at seasonal scales. Therefore, from a seasonal perspective it is interesting to compare these events at continental scales. Herein, surface temperature analysis data (23) and multiproxy surface air temperature reconstructions since 1500 (11) are used to place these recent extreme European summers in a palaeoclimatic context (15). Figure 2 displays the European mean summer land surface air temperature distribution for the 1500–2010 period. The European mean 2010 summer [temperature anomaly (ΔT) = +1.8°C, 3.5 SDs relative to 1970–1999] was ~0.2°C warmer than the previous warmest summer of 2003 (11). This number is even more noticeable if we consider that the European average temperature is defined for the land area [35°N, 70°N] and [25°W, 40°E], thus excluding many regions affected by outstanding temperatures in 2010. Further, the historical evolution of the hottest summers in Europe (Fig. 2, bottom) suggests that the last decade stands substantially above any other 10-year period since 1500. Taking into account the uncertainties in the reconstruction (11, 15), we found that at least two summers in this decade have most likely been the warmest of the last 510 years in Europe.

Figure 3 further stresses the exceptional magnitude of the 2010 summer, displaying the amplitude

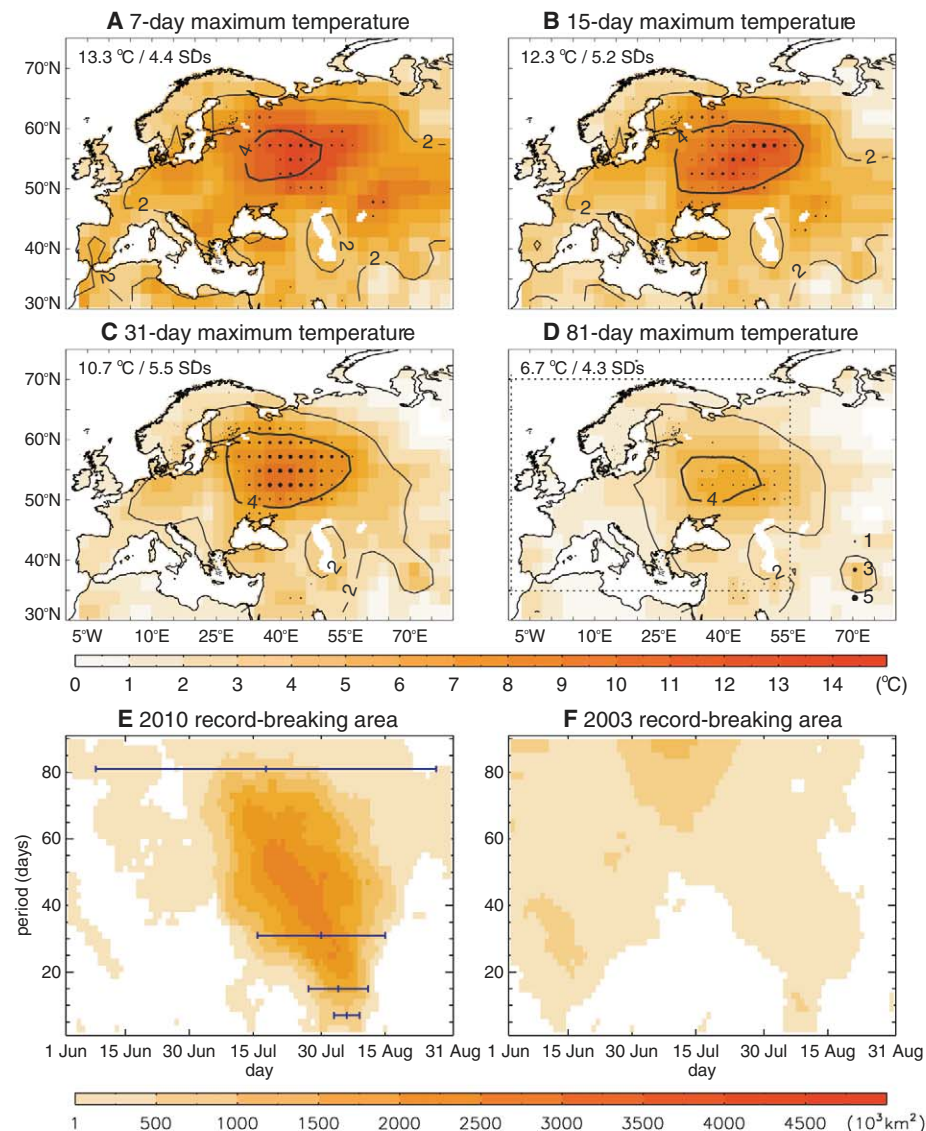


Fig. 2. European summer temperatures for 1500–2010. Statistical frequency distribution of best-guess reconstructed and instrument-based European ([35°N, 70°N], [25°W, 40°E]) summer land temperature anomalies (degrees Celsius, relative to the 1970–1999 period) for the 1500–2010 period (vertical lines). The five warmest and coldest summers are highlighted. Gray bars represent the distribution for the 1500–2002 period (11), with a Gaussian fit in black. Data for the 2003–2010 period are from (23). (Bottom) The running decadal frequency of extreme summers, defined as those with temperature above the 95th percentile of the 1500–2002 distribution. A 10-year smoothing is applied. Dotted line shows the 95th percentile of the distribution of maximum decadal values that would be expected by random chance (15).

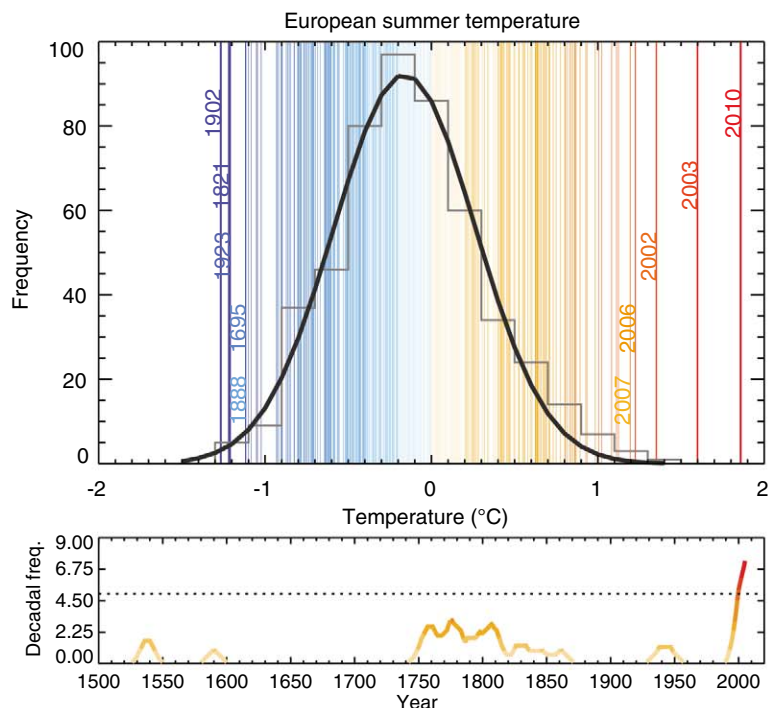
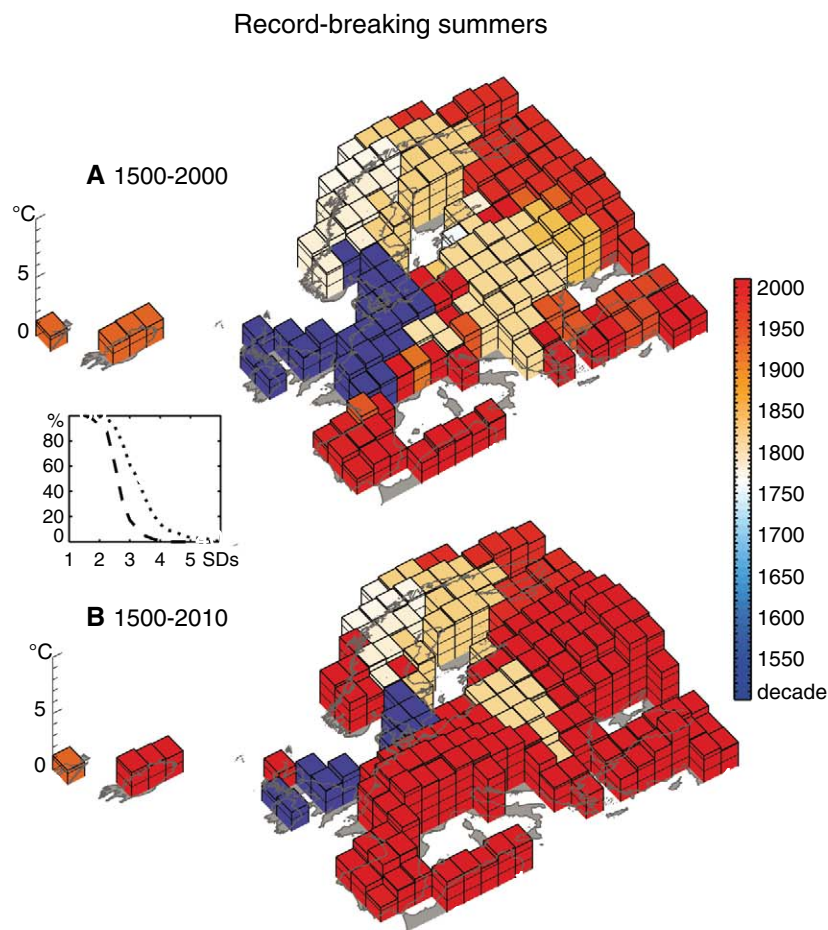


Fig. 3. Spatial distribution of the hottest European summers (24). The height and the color of the bars indicate the best-guess maximum anomaly (degrees Celsius, relative to the 1970–1999 period) and the decade of the corresponding summer, respectively, for the periods (A) 1500–2000 and (B) 1500–2010. For better readability, each bar is subdivided with 1°C intervals. The embedded plot shows the corresponding percentage of European areas with summer maxima above the given temperature (in SDs) for the 1500–2000 (dashed line) and 1500–2010 (dotted line) periods. Data sources are (11) (1500–2002) and (23) (2003–2010).

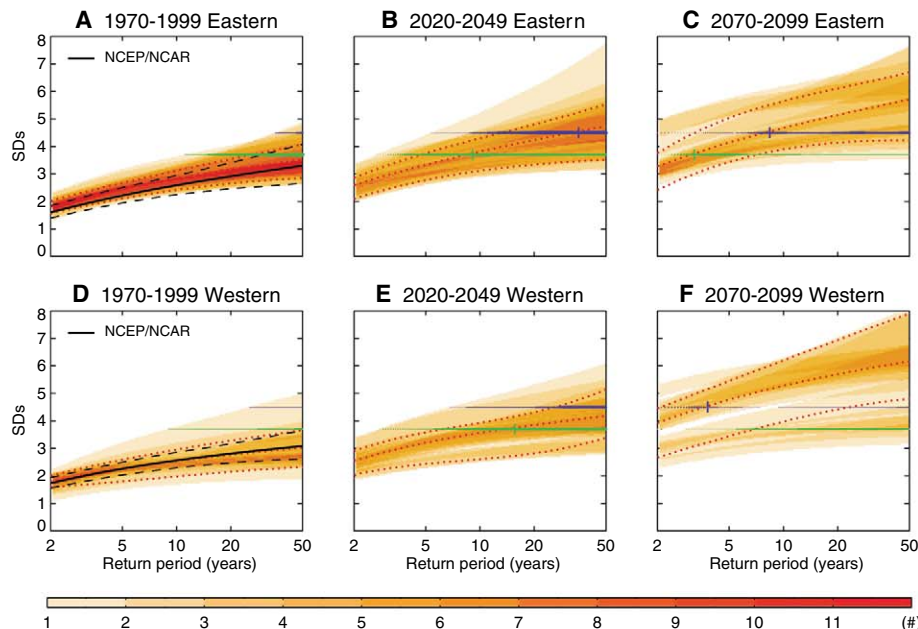


of the hottest summers across Europe and the decade when they occurred (24). To highlight the contribution of summers in the 2001–2010 dec-

ade, the analysis was initially restricted to the 1500–2000 period (Fig. 3A) and then updated to 2010 (Fig. 3B). Until the end of the 20th century

(20C), maximum seasonal temperatures across Europe mostly ranged 2 to 3 SDs of their 1970–1999 climatology, with regional extreme summers

Fig. 4. Multi-model projections of mega-heatwaves. Return periods (years) of 7-day summer maximum regional temperature for three 30-year periods and 11 RCMs forced with A1B emission scenario (26) over (A to C) EE ([52.5°N, 60°N]; [27.5°E, 40°E]) and (D to F) WE ([42.5°N, 50°N]; [0°E, 15°E]). Anomalies are depicted as SDs of the control period 1970–1999. Shading indicates the degree of agreement between models as measured by the overlapping of their 95% confidence interval limits. Dotted lines comprise the 75% density distribution, with the median in between. Black lines represent the corresponding fit from reanalysis (16). Blue and green dots represent the RCM-projected distribution of RPs for a 2010- and a 2003-like event, respectively, with the size proportional to the level of model agreement. The vertical mark indicates the best guess, as obtained from the median of the RCM distribution.



clustering in a few decades of the last five centuries. During the 2001–2010 decade, 500-year-long records were likely broken over ~65% of Europe, including eastern Europe (2010), southwestern-central Europe (2003), the Balkans (2007), and Turkey (2001). These summers have considerably contributed to the upper tail of the European distribution of summer maxima (Fig. 3, inset). Thus, the percentage of European regions with seasonal maxima above 3 SDs (>99th percentile of the 1970–1999 distribution) has doubled within one decade. The 2003 and 2010 summers were likely the warmest on record over ~25% of Europe, standing as major contributors to the current European map of the hottest summers.

It is noticeable that the two hottest summers in Europe resulted from subseasonal heatwaves of outstanding magnitude and large spatial extent. This raises the question of whether these “mega-heatwaves” (25) (and regional extreme events at other time scales) will become more frequent in the future. To address this question, we evaluated transient experiments from 11 high-resolution regional climate models (RCMs) driven with different general circulation models (GCMs), which are forced with the A1B emission scenario (15, 26). The analysis emphasizes analogs of the 2010 and 2003 events over the eastern (EE) and western (WE) European regions that were strongly affected by these mega-heatwaves (fig. S11).

Anthropogenic changes are assessed in terms of return periods (RPs) of maximum 7-day summer regional temperature for three time slices (1970–1999, 2020–2049, and 2070–2099) (Fig. 4). Regional mean temperatures were normalized with reference to the 1970–1999 climatology and hereafter expressed as SDs (27). Simulations for the 1970–1999 period indicate a reasonable model skill, although there is a considerable spread of model results, particularly for long RPs (figs. S12 and S13). The ensemble of RCMs projects that

weekly heat spells of the magnitude of the second week of August 2003 (7-day anomaly of 3.7 SDs), which are extremely rare in the 20C simulations, will probably occur in 2020–2049, with a best-guess RP of ~10 years in EE and ~15 years in WE. However, a weekly 2010-like event (~4.5 SDs) remains very rare in the same period (best-guess RPs of >30-year over both regions). By the end of the 21st century (21C), such extreme weekly heat spells are expected every ~8 years in EE and ~4 years in WE, whereas some models show regular 2003-like anomalies (about every second summer). The estimated RPs involve major model uncertainties and should be carefully interpreted, given the high natural variability of such extreme events. Thus, some RCMs show several events similar to 2010 in the period 2020–2049 (fig. S14, left), whereas others show just one event similar to 2003 within the last 30 years of the 21C (fig. S14, right).

The increase in probability of 2003- and 2010-type events depends on the time scale addressed and differs if the seasonal anomaly is emphasized instead of the weekly time scale above (SOM text). The analysis of RCM time series for the 2011–2100 period reveals 2003 analogs (at all 7- to 91-day temporal scales) before 2050 in more than half of the models (figs. S14 and S15). However, the same cannot be stated for a 2010 analog until the second half of the 21C, particularly at monthly and seasonal scales. For the last 30 years of the 21C, the occurrence of 2010-like monthly anomalies (~5 SDs) increases rapidly to one event per decade in most models, and by 2100 all models present at least one summer like 2010.

The enhanced frequency for small to moderate anomalies of 2 to 3 SDs is mostly accounted for by a shift in mean summer temperatures (compare Fig. 4 with fig. S18). However, the future probabilities of mega-heatwaves with SDs sim-

ilar to 2003 and 2010 are substantially amplified by enhanced variability. Particularly in WE, variability has been suggested to increase at inter-annual and intraseasonal time scales (1, 2) as a result of increased land-atmosphere coupling (28) and changes in the surface energy and water budget (2, 29). Models indicate that the structure of circulation anomalies associated with mega-heatwaves remains essentially unchanged in the future (SOM text).

Our results reveal that along with the reported changes in local heatwaves (8), there is an increasing likelihood of mega-heatwaves over highly populated areas of Europe with magnitudes such that they would exceed the exceptional current weekly-to-seasonal temperature maxima of WE within the next four decades and of EE afterwards. Given the disastrous effects of the 2003 and 2010 events, these results venture serious risks of simultaneous adverse impacts over large areas if no adaptive strategies are adopted.

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25. The concept of mega-heatwave is herein used to refer to regional mean temperature anomalies (over ~1 million km²) of extraordinary amplitude (approximately ≥ 3 SDs relative to the 1970–1999 period) at subseasonal scales (of at least 7 days), thus differing from the classic local heatwave definition.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1201224/DC1
Materials and Methods
SOM Text
Figs. S1 to S18
Table S1
References and Notes

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¹³C NMR Guides Rational Design of Nanocatalysts via Chemisorption Evaluation in Liquid Phase

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The search for more efficient heterogeneous catalysts remains critical to the chemical industry. The Sabatier principle of maximizing catalytic activity by optimizing the adsorption energy of the substrate molecule could offer pivotal guidance to otherwise random screenings. Here we show that the chemical shift value of an adsorbate (formic acid) on metal colloid catalysts measured by ¹³C nuclear magnetic resonance (NMR) spectroscopy in aqueous suspension constitutes a simple experimental descriptor for adsorption strength. Avoiding direct contact between the ¹³C atom and the metal surface eliminates peak broadening that has confounded prior efforts to establish such correlations. The data can guide rational design of improved catalysts, as demonstrated here for the cases of formic acid decomposition and formic acid electro-oxidation reactions.

In 1913, the French chemist Paul Sabatier introduced a qualitative concept in chemical catalysis that described the appropriate interaction between catalyst and substrate as a balance between extremes. If the interaction is too weak, the substrate will fail to bind to the catalyst and no reaction will take place; if it is too strong, the catalyst will be blocked by the substrate, intermediate, or product, hindering turnover of the catalytic cycle (1). The Sabatier principle is best illustrated by Balandin's volcano relations between reaction rates and adsorption energies, which

was employed from the study of catalytic decomposition of formic acid over transition metals by Sachtlar and Fahrenfort in 1961 (2). However, various thermodynamic data, such as the heat of formate or oxide formation, were actually used to estimate the adsorption energies in these early studies. These bulk thermodynamic properties established under different reaction conditions were not the best descriptors for surface adsorption structures and energies (3). Over the past decade, theoretical calculation of adsorption energies on solid surfaces using density functional theory (DFT) has become practical on account of enhanced computing power, although data to validate this complex modeling under realistic reaction conditions [in liquid phase or under pressure, rather than in ultrahigh vacuum (UHV)] have been scarce (4). In addition, Somorjai earlier demonstrated that face specific-

ity is a characteristic property of adsorption (5), which implies that the modeling of working catalysts that comprise different surfaces while taking complexities such as solvent effects, surface specificity, and adsorbate-adsorbate interactions into account may not be a simple task.

Traditional resonance methods such as electron spin resonance (ESR) and nuclear magnetic resonance (NMR) can yield a wealth of information about the electronic interactions between atoms under non-UHV conditions in the liquid or solid phase (6, 7). Studies of chemisorption of small probing molecules such as CO and ethylene on metal surfaces by both solution and solid-phase NMR have been reported (8–13). A broad NMR peak was observed because of particle anisotropy and magnetic field inhomogeneity. Newmark (12) and Bradley (13) used solution-phase ¹³C NMR to probe adsorption of ¹³CO onto different metal colloids in solution. They showed that the tumbling motion of nano-sized metal particles in solution is sufficiently fast to reduce the particle anisotropy in a magnetic field over the liquid suspension of particles. However, all of the above works encountered significant Knight-shift effects in their NMR peaks due to the coupling of conduction electrons from the Fermi levels of the metal particles with the probe ¹³C atom in direct contact, which severely perturbed and broadened the ¹³C chemical shift values [peak position and peak width are affected, ranging from a few to hundreds of parts per million (ppm)]. This problem precluded the potential use of chemical shift values for assessing adsorption strength until our initial work on the adsorption of formic acid on Ru nanoparticles. We showed that the presence of the O spacer atoms in the adsorbed formate substantially reduces the Knight-shift effect on the ¹³C nucleus, because it is not directly coupled to the

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metal surface. This enabled the observation of sharp adsorption peaks on nanosized Ru metal (14).

Here we show that the unequal sharing of bonding electrons around the ^{13}C nucleus of the adsorbate molecule on the metal surfaces gives rise to variations in ^{13}C chemical shift values, which correlate to the adsorption states. We found that the measured chemical shift of formic acid on various surfaces shows a direct linear relationship with those surfaces' catalytic activity for formic acid decomposition in water, which can be used to guide the identification of active catalytic phases. The chemical shift value is also dependent on the type and size of metal: Electron-rich metals (such as Au, Ag, and Pd) show a greater particle size effect on their chemical reactivity than relatively electron-poor metals (Ru shows no size dependence on chemical reactivity). In addition, adsorbed formate is also considered an important intermediate on metal catalysts in formic acid electro-oxidation (to CO_2 and H^+) in direct formic acid fuel cells (DFAFCs). Thus, the chemical shift of the adsorbed formate is used to guide rational catalyst design for this important reaction, leading to new Ag core–Pd shell catalysts. These materials show superior activity to that of catalysts previously reported in the literature.

We began by acquiring static ^{13}C NMR spectra of formic acid in D_2O suspensions of polyvinylpyrrolidone (PVP)–stabilized Pd nanoparticles (15). We observed four resonance peaks (Fig. 1A). The typical peak width was narrow (about 0.02 to 0.04 ppm) but was larger than those recorded for dissolved gas and molecular species, reflecting the slower relaxation associated with surface-bound states. The resonance with the highest intensity at 165.89 ppm was assigned to weakly adsorbed formic acid in exchange with the free form in solution (a slightly upfield shift as compared to the free molecule at 165.58 ppm). The intensity of this resonance decreased during the

reaction, which indicated the gradual consumption (decomposition) of the formic acid. Based on the differences in the electronic environments of the carbon nuclei (the degree of back-bonding), the three other resonances at 165.42, 165.69, and 165.95 ppm were assigned to three different adsorbed modes closely related to monodentate, multimonomdentate, and bridging formate species [see the Fourier transform infrared spectroscopy (FTIR) spectra in fig. S3]. The same assignments were adopted in the case of Ru, consistent with the general order in the observed values of the corresponding molecular Ru formate complexes from NMR and IR (14). Figure 1B shows that an increase in Pd particle size from 2.3 to 5.2 nm results in increased ratios of bridging to multimonomdentate and to monodentate species. This result, along with similar observations of adsorbed formate species on Ru (Fig. 1C) and other types of metallic particles, is consistent with the geometric model based on statistical analysis of the progressive change in terrace, edge, and corner sites on different sizes of close-packed crystallites (16).

We showed that the ^{13}C with an O spacer in formate species gives rise to sharp adsorption peaks. This contrasts with the broad peaks observed from ^{13}C atoms in direct contact with metals. The calculated local density of states (LDOS) at various energies around the ^{13}C of bridging formate adsorbed on Pd clusters modeled by DFT showed a significant degree of overlap of the 2s and 2p orbitals of ^{13}C with metal d-electrons, despite separation by the O atom spacer (fig. S12 and table S3). Therefore, the chemical bonding information is retained through the two-atom distance but with a much reduced Knight-shift effect (17, 18) [supporting online material (SOM) text].

We then proceeded to acquire analogous spectra using Rh, Pt, Ag, and Au particles. Figure 2A compares the chemical shifts of bridging formate species obtained over these different transition metal

colloids. The chemical shift value increases from electron-rich metals (weak adsorption) to comparatively electron-poor metals (strong adsorption) with the same face-centered cubic (fcc) structure [Ru with hexagonal close-packed (hcp) structure was not included]. We used the DFT-derived d-band center model to account for this trend, which has been successfully used in the literature to model various chemisorption systems, in which the closer the d-band center is to the Fermi level (toward zero), the higher the obtained adsorption energy (19). Thus, the plot in Fig. 2A is consistent with the conclusion that the chemical shift value of the noncontact ^{13}C in the formate species indeed reflects the strength of chemisorption. As shown in Fig. 2B, the rates of formic acid decomposition to CO_2 and H_2 at room temperature (the loss of formic acid and production of CO_2 were monitored in situ by NMR) over these metals also show a linear relationship with chemical shift values (the catalytic activity of Pt was slightly lower than expected due to surface fouling/poisoning). This linear relationship matches well with the order of transition metals on the left hand of the original volcano published by Sachtler and Fahrenfort in 1961 (2), which suggests that the rate of reaction is clearly related to the stability of the transition state of the adsorbed formic acid before CO_2/H_2 formation on the metal surface: The stronger the dynamic adsorption, the higher the activity, as described by the Sabatier principle.

Among the transition metals studied, Pt is well established in the literature to be the most active for formic acid decomposition in the gas phase (3); however, Pd has been reported to be the most active for formic acid decomposition and electro-oxidation in the aqueous phase (20). We observed the order $\text{Pd} > \text{Rh} > \text{Pt} > \text{Au} > \text{Ag}$ for both chemical shift and formic acid decomposition activity in water when ~2- to 4-nm particles

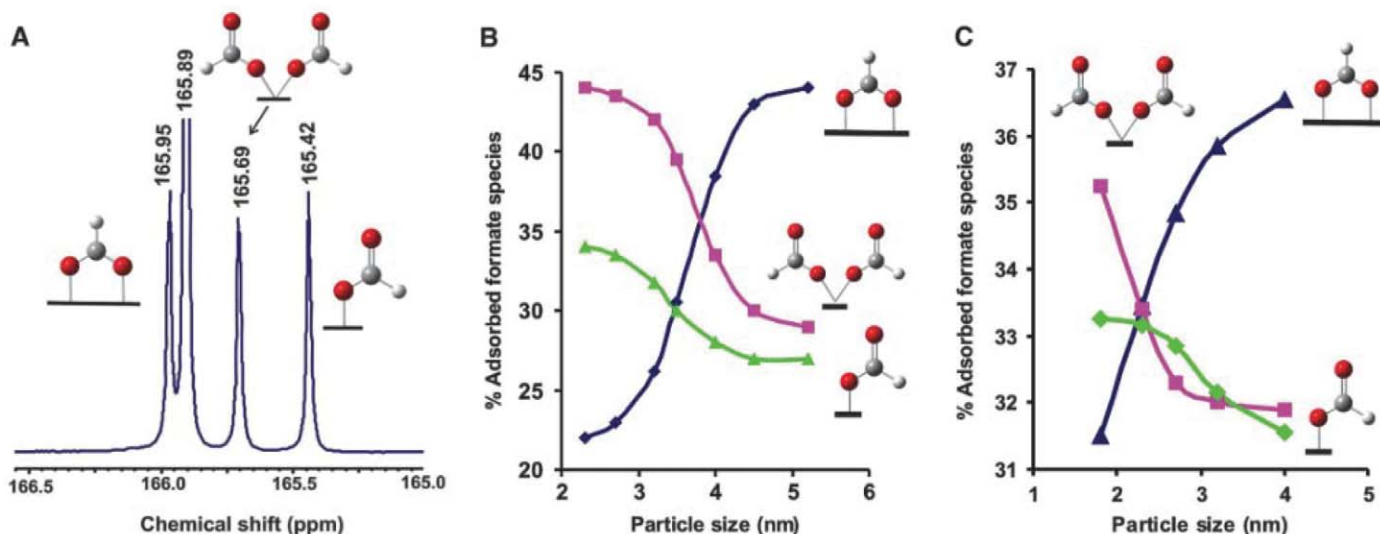


Fig. 1. (A) ^{13}C NMR spectrum of formic acid and formate adsorbed on PVP-Pd nanoparticles (4.5 nm in diameter). (B) The relationship between the percent of formate species in each mode of adsorption and the size of the PVP-Pd

nanoparticles. (C) The relationship between the percent of formate species in each mode of adsorption and the size of the PVP-Ru nanoparticles (pink, multimonomdentate; blue, bridging; green, monodentate formates).

were used (except for the Ag particles, which were 8 nm in diameter). This order does not match that of calculated static adsorption energies of bridging formate on (100), (110), and (111) metal surfaces (table S1 and fig. S9), using first-principles DFT calculations, implying that the formation of bridging species on the surface is not the rate-determining step in this reaction. On the other hand, Fig. 2C shows the calculated H adsorption energies on (111) metal surfaces, which appear to agree with our experimental order. We therefore propose that H transfer from the adsorbed intermediate to the surface is the key step for the formic acid dissociation into CO₂ and H₂. Regarding the mechanism of formic acid decomposition to CO₂ and H₂ on metal surfaces, the initial O-H scission is a facile reaction, but further C-H scission in adsorbed formate to CO₂ and H has been shown to be rate-limiting over Pd (110), using temperature-programmed desorption and x-ray photoelectron spectroscopy experiments (21). We therefore envisage that the metal surface not only adsorbs the formate species through the interaction of the O atoms in a static bridging mode, but also facilitates C-H scission in the transition state, probably with the involvement of water molecules. Bearing in mind the long time scale of

NMR, the chemical shift value can reflect the global interactions of metal-OC and metal-HC species before product formation. Thus, the chemical shift value we measured can clearly reflect the global adsorption energy of the dynamic adsorption states over the metal particle surfaces in water under the reaction conditions. This information obtained is in clear contrast with the traditional approach of calculating adsorbed structure and enthalpy of the stable formate from specific crystallographic faces, based on energy minimization of data from UHV experiments (table S1 and fig. S9), which fails to reflect the global interactions of the molecule on the metal surface along the reaction trajectory. The solvent (water) effect on formic acid oxidation over a metal surface has recently been stressed in a modeling approach (22, 23). The authors highlighted the importance of the local aqueous environment on C-H scission kinetics of formic acid on a metal surface (23).

Regarding catalyst design, it is important to emphasize that the global adsorption energy depends not only on the type of metal but also on the size, shape, surface features, and local factors such as coverage, dipole interactions, and solvent effects. Chemical shift values may also reflect these effects and thereby lead to optimization

of the respective parameters. Particle size has proven especially influential for Au nanoparticles, which show exceptional activity at sizes below 3 nm (24). We therefore examined whether the chemical shift value of adsorbed formic acid depends on the number of metal atoms in the catalyst particle. The size effect was studied over some selected metal colloids with narrow size (diameter) distributions (± 0.5 nm). Figure 3, A and B, clearly show that decreasing the particle size for most metal nanoparticles indeed results in a more deshielded chemical shift, indicating stronger adsorption of formic acid. It is generally accepted that the increase in the proportion of lower coordination sites at smaller particle size accounts for the increase in adsorption energy. However, it is surprising to observe that the degree of change is actually critically dependent on the type of metal used. Over a similar size range, electron-rich Au shows the greatest size effect, followed by Pd and Pt, which show moderate changes in chemical shift values upon decreasing particle size. Figure 3C shows a higher rate of formic acid decomposition to CO₂ at smaller Au particle sizes (higher chemical shift values). In contrast, the most electron-poor metal we studied, Ru, shows almost no size dependence. The exact origin of the size dependence activity is not

Fig. 2. Plots of (A) measured ¹³C chemical shift of adsorbed bridging formate over different metal colloids with fcc structure; (B) the correlation of chemical shift with the rate of formic acid decomposition; and (C) calculated adsorption energies of H on different fcc (111) metal surfaces. The error bars represent the average values obtained for five repeated measurements with reference to highest and lowest values.

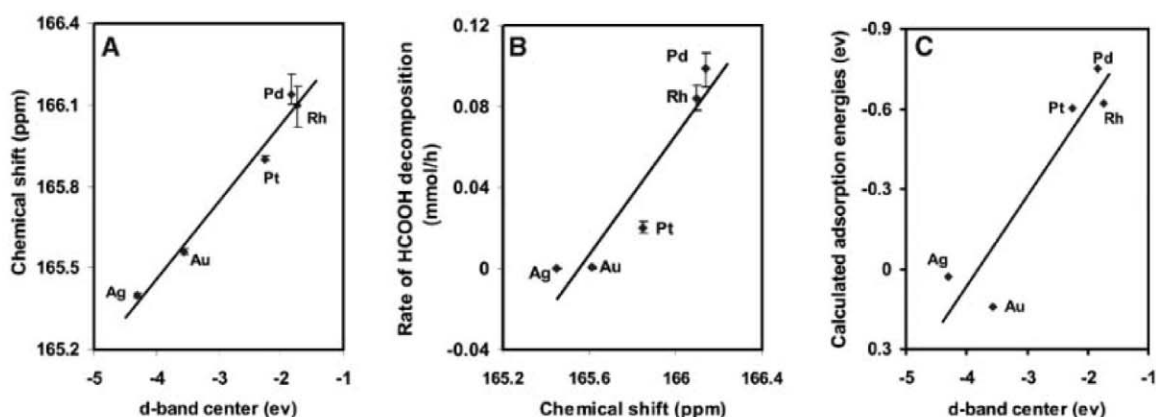
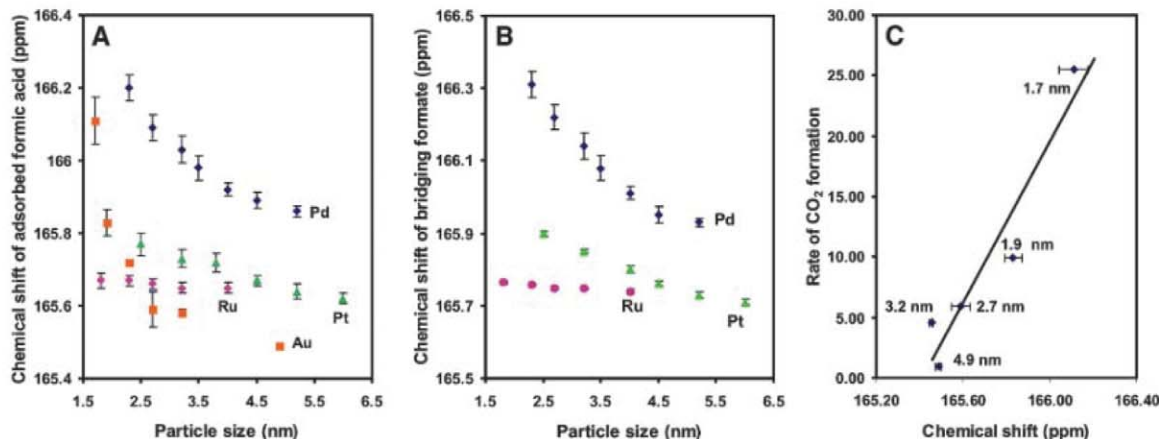
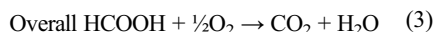
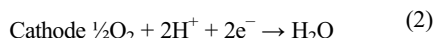


Fig. 3. Correlations between particle sizes and chemical shifts of adsorbed (A) formic acid in exchange with the free form and (B) bridging formate. (C) Correlation between ¹³C chemical shift of adsorbed formic acid with initial rate of CO₂ formation over different sizes of Au nanoparticles. The error bars represent the average values obtained for five repeated measurements with reference to highest and lowest values.



yet known, and our measurements cannot be explained by the change in coordination number based on the Benfield geometric model (25). On the other hand, the electronic structure (positions of Fermi level, d-band center, and degree of d-band filling) of the type of metal with respect to energy levels of surface bonding and antibonding orbitals of adsorbate appears to play a more dominant role in giving the overall size effect (see SOM text for rationalization of particle size effects).

The data clearly suggest that the small but diagnostic chemical shift value of nondirect ^{13}C atoms on a metal colloid can be used to represent the global adsorption energy under real catalytic conditions in the liquid phase. The small degree of peak shift (0.1 to 1 ppm) with peak width of <0.02 ppm in our solution-phase NMR spectra would be unobservable if the probing atoms were in direct contact with the metal, thus causing severe Knight shift and peak broadening. We next sought to make use of this capability to guide the design of more effective catalysts. We chose to focus on direct DFAFCs, a promising alternative clean energy sources for portable devices (26). The electrochemical reactions in a DFAFC are shown below (Eqs. 1 to 3)



The anode reaction is very similar to the catalytic decomposition of HCOOH to CO_2 , with C-H scission as the rate-limiting step (27), but in this case, protons are formed instead of H_2 gas under anodic current. We therefore deposited the same monometallic nanocatalysts (colloids) on high-surface-area Vulcan C. After the removal of the stabilizer, which resulted in no apparent re-

construction (figs. S6 and S7), the materials were tested by cyclic voltammetry (Fig. 4B) and showed the same metal activity ranking as in suspension (Fig. 2B). Small Pd particles supported on Vulcan C again proved the most active. It is clear from Fig. 2 that increasing electronic density beyond Pd may further enhance the adsorption of formate, leading to the production of CO_2 and H^+ under electrochemical conditions. In order to retain the Pd surface, which was shown to be the most active among the studied monometallic elements, methods for electron promotion, including doping, lattice substitution, and support addition, can be considered. It has been reported that the use of a core-shell configuration, with the core of higher Fermi level to directly donate electron density to the shell while keeping the external surface identical, can promote electroreduction of O in polymer electrolyte membrane fuel cells (28, 29). We anticipated that stronger back-donation to the formate from the electronically enriched Pd would be characterized by an increased chemical shift of the Pd shell through liquid ^{13}C NMR evaluation. Therefore, as a proof of concept, we prepared a series of bimetallic metal-core Pd-shell nanocatalysts ($\text{M}@\text{Pd}$, where $\text{M} = \text{Ru}, \text{Rh}, \text{Pt}, \text{Ag}$, and Au) and characterized them by NMR. These core-shell nanoparticle catalysts were synthesized by controlled colloidal chemistry with a good degree of standardization in structure at the atomic scale, to allow attribution of the changes in adsorption to the type of core metal used. The electronic properties of the core-shell catalyst colloids were inferred by analyzing the chemical shifts of adsorbed bridging formate in solution, and the catalytic activity for formic acid electro-oxidation was examined over the solid C-supported bimetallic particles after the removal of stabilizer. Before this experiment, CO chemisorption-FTIR and surface plasmon techniques (for cases of Au or Ag cores) clearly confirmed that the Pd-shell

layers were fully covering the inner core metal (no CO chemisorption peaks were evident from exposed metal) over the 1:1 $\text{M}@\text{Pd}$ samples. The electronic enrichment effect of metal core to Pd shell to further enhance the chemical shift value was clearly evident in terms of the relative work functions of the metals. An excellent linear correlation was indeed observed as shown in Fig. 4A.

The work function of a metal is the minimum energy needed to remove an electron from the Fermi level. It is similar to the ionization potential (binding energy) but reflects the energy state of electrons in a solid. It is clear from Fig. 4A that there is a charge transfer between the metal core and metal shell due to a difference in work functions, which affects the dynamic adsorption strength (change in chemical shift value) under the same catalytic operation conditions in liquid phase. It may be argued that there is also a lattice mismatch between the Pd shell and the underlying metal, causing an isomorphous (structural) effect on the electronic structure of Pd. However, the excellent linear correlation clearly indicates that the charge transfer between the core metal and Pd shells of these bimetallic nanoparticles appears to play a more dominant role (19) in determining the dynamic adsorption energy of formate. Furthermore, an excellent linear relationship between chemical shift and the specific activity of electrocatalysts is again observed (Fig. 4B). From this limited rational screening exercise, $\text{Ag}@\text{Pd}$ offers the highest specific activity for all samples guided by the chemical shift study; the activity of this electrocatalyst is more than 30% higher than that of $\text{Au}@\text{Pd}$, which is among the best reported electro-catalysts for formic acid oxidation (30). We must stress that the focus of this paper is not primarily the development of formic acid fuel cell technology. The main challenges facing formic acid fuel cell catalysts extend beyond activity to stability and durability (27, 31), which were not addressed in this work. However, we are confident that more generally, nanocatalysts can be easily screened by NMR for rapid chemisorption evaluation (typically in few minutes) using our present methodology, without the need to engage in a more complex testing procedure.

These chemisorption screenings contrast with the direct evaluation of catalytic activity and selectivity under reaction conditions by high-throughput approaches, wherein the catalytic performance could potentially miss essential information. For example, strongly chemisorbed substrates may not show high activity, but with the knowledge of chemisorption strength gained by NMR screening, catalytic properties can be rationally tuned for improvement. Similarly, careful differentiation of NMR adsorption peaks allows identification of the active phase(s) in a complex catalyst mixture, otherwise obscured in a gross activity/selectivity evaluation. Although our method at present requires a spacer atom bonded to ^{13}C to ameliorate the Knight-shift effect, this is fortunately possible in many real substrate molecules of interest, such as glycerol, ethylene glycol, and

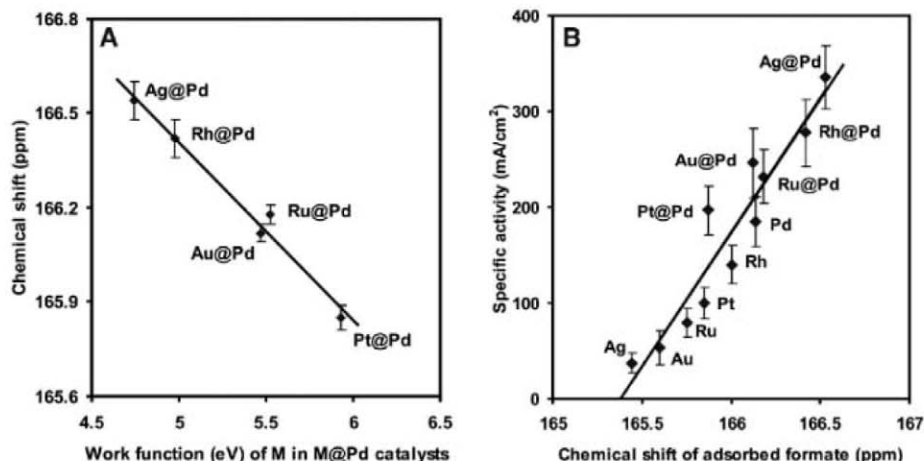


Fig. 4. (A) Relationship between ^{13}C chemical shifts of adsorbed bridging formates on $\text{Ag}@\text{Pd}$, $\text{Rh}@\text{Pd}$, $\text{Au}@\text{Pd}$, $\text{Ru}@\text{Pd}$, and $\text{Pt}@\text{Pd}$ bimetallics and work functions of (111) fcc Ag, Rh, Au, Ru, Pd, and (0001) hcp Ru. (B) Correlation between chemical shift and specific activity (at 0.2 V) of both C-supported monometallic and core-shell bimetallic catalysts for formic acid electro-oxidation. The error bars represent the average values obtained for five repeated measurements with reference to highest and lowest values.

ethanol in the biomass conversion sector, to name but a few examples.

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Oriented 2D Covalent Organic Framework Thin Films on Single-Layer Graphene

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Covalent organic frameworks (COFs), in which molecular building blocks form robust microporous networks, are usually synthesized as insoluble and unprocessable powders. We have grown two-dimensional (2D) COF films on single-layer graphene (SLG) under operationally simple solvothermal conditions. The layered films stack normal to the SLG surface and show improved crystallinity compared with COF powders. We used SLG surfaces supported on copper, silicon carbide, and transparent fused silica (SiO₂) substrates, enabling optical spectroscopy of COFs in transmission mode. Three chemically distinct COF films grown on SLG exhibit similar vertical alignment and long-range order, and two of these are of interest for organic electronic devices for which thin-film formation is a prerequisite for characterizing their optoelectronic properties.

Methods for crystallizing organic subunits into two-dimensional (2D) and three-dimensional (3D) covalent organic frameworks (COFs) remain in their infancy (1–4). COFs organize molecular components into periodic networks linked by covalent bonds, providing predictable structures with long-range order that is usually only found in noncovalent assemblies. These materials exhibit many desirable properties, including outstanding thermal stability, permanent porosity with high specific surface area, and the lowest densities of any organic material (5). However, the frameworks are inherently cross-linked and insoluble and are produced as

either microcrystalline powders from solvothermal reactions or submonolayers by sublimation of the monomers onto crystalline metal surfaces (6–9). The limited utility of these forms precludes many applications for COFs. For example, 2D layered COFs incorporate functional π -electron systems into ordered structures ideally suited for optoelectronic devices (10–13). As unprocessable powders, these materials cannot be interfaced reliably to electrodes or incorporated into devices to harness or even quantify these properties. We now report the synthesis of oriented 2D layered COF films on single-layer graphene (SLG) surfaces.

The notable optical, electronic, and mechanical properties of SLG have attracted considerable interest, for example, as a possible replacement for tin-doped indium oxide transparent electrodes (14, 15). SLG's 2D, atomically precise structure is also well suited for interfacing to 2D layered networks. Large-scale graphene synthesis by metal-based chemical vapor deposition (CVD) has advanced dramatically in recent years (16–19), including

the high-throughput growth of 76-cm-wide samples supported on plastic substrates (20). We demonstrate that oriented COF films form under operationally simple solvothermal conditions on SLG supported by several different substrate materials: polycrystalline Cu films on Si wafers (SLG/Cu), fused SiO₂ (SLG/SiO₂), and SiC (SLG/SiC). The COF films are crystalline and oriented with their aromatic groups stacked perpendicular to the SLG surface on each substrate. Three boronate ester-linked COFs have been crystallized as thin films, including a square Ni phthalocyanine lattice of interest for organic photovoltaic devices (12, 13).

The solvothermal condensation of 1,4-phenylenebis(boronic acid) (PBBA) and 2,3,6,7,10,11-hexahydroxytriphenylene (HHTP) in a mixture of mesitylene:dioxane (1:1 v/v) at 90°C in the presence of SLG/Cu forms a framework known as COF-5 (Fig. 1), as both an insoluble powder and as a continuous film on the graphene surface (21). Powder x-ray diffraction (PXRD) (fig. S1) and Fourier transform infrared spectroscopy of the unpurified powders (fig. S2) indicate that crystalline COF-5 is obtained with only minor amounts of residual reactants in as little as 1 hour (fig. S3), faster than the 72-hour reaction time used for its discovery (1). These observations prompted us to investigate whether graphene catalyzes COF-5 powder formation, but we obtained similar results in the absence of SLG (fig. S4). We conclude that long reaction times are not always necessary to produce COFs, suggesting that their films might be incorporated into devices more rapidly than previously thought.

We used synchrotron x-ray diffraction to compare the crystallinity of the COF-5 films and powders. Figure 2, A and B, shows 2D x-ray diffraction patterns obtained from a powder sample and a film grown on SLG/Cu, respectively, using identical incident beam and scan parameters. The data in Fig. 2A were collected in transmission mode by suspending a ~0.1-mm-thick powder sample perpendicular to the incident beam.

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The Bragg peaks in Fig. 2A appear as rings because of the random orientation of grains in the sample (see inset). For Fig. 2B, as well as all subsequent diffraction data obtained from films, we used grazing incidence diffraction (GID), in which the substrate surface is horizontal and nearly parallel to the incident beam. Axes labels $Q_{\perp}$ and $Q_{\parallel}$ (where hQ is the momentum transfer of a scattered photon, and h is Planck's constant h

divided by 2π) are defined using the convention $Q_{\perp} = 4\pi/\lambda \sin(\delta/2)$ and $Q_{\parallel} = 4\pi/\lambda \sin(\nu/2)$, where λ is the x-ray wavelength, and δ and ν are the vertical and horizontal scattering angles, respectively (22). In contrast to Fig. 2A, the scattered intensity in Fig. 2B is concentrated near $Q_{\perp} = 0$, indicating that grains in the film exhibit fiber texture. Their c -axis orientations are centered on the surface normal, but they are randomly rotated about this axis

(see inset). Projections of these data sets near $Q_{\perp} = 0$ (Fig. 2C) indicate peaks from both samples at 0.24, 0.42, 0.48, 0.64, 0.84, and 0.88 $\text{\AA}^{-1}$, corresponding to 100, 110, 200, 210, 220, and 310 Bragg peaks of a hexagonal lattice with in-plane lattice parameters $a = b = 29.9$ $\text{\AA}$, extremely close to the calculated (30.0 $\text{\AA}$) and measured (29.7 $\text{\AA}$) values previously reported for COF-5 powders (1). The concentration of these peaks near $Q_{\perp} = 0$ in the film shows that the hexagonal lattice of the COF-5 grains is aligned parallel to the substrate surface.

Figure 2C highlights additional peaks not shared by both samples. First, the film exhibits diffraction peaks at 0.97, 1.06, 1.21, and 1.27 $\text{\AA}^{-1}$ that are not present in Fig. 2A or in reported PXRD of COF-5 powder. These peaks correspond to the COF-5 400, 320, 500, and overlapping 330 and 420 Bragg peaks. Additionally, the 200 peak (at 0.48 $\text{\AA}^{-1}$) is attenuated in the film compared with the powder. This difference can arise from trace impurities in the pores or from subtle differences in the horizontal offset between layers in the film compared with the powder (23). Powder rings in Fig. 2A at 1.27, 1.68, 1.87, and 1.94 $\text{\AA}^{-1}$ correspond to Bragg peaks from residual starting materials trapped in the pores of the unpurified powder samples. The broad powder ring in Fig. 2A centered at 1.83 $\text{\AA}^{-1}$ corresponds to the 001 Bragg peak and indicates that the stacked COF-5 sheets are in van der Waals contact (with an out-of-plane lattice parameter $c = 3.43$ $\text{\AA}$). This peak is absent in Fig. 2B because the c axes of grains in the film are oriented perpendicular to the substrate. Instead, the 001 peak of the film is observed (Fig. 2D) as a diffuse arc of scattering centered at $Q_{\perp} = 1.85$ $\text{\AA}^{-1}$ by obtaining additional measurements near $Q_{\parallel} = 0$ and a large out-of-plane diffraction angle, corresponding to large $Q_{\perp}$. The width of this peak in $Q_{\parallel}$ provides a rough measure of the orientational order in the film (24) and indicates (see supporting online material text) that most grains orient their c axes within $\pm 13^\circ$ of the surface normal (fig. S5). Accounting for instrumental resolution and assuming platelet-shaped grains (25), Debye-Scherrer analysis of Fig. 2, B and D, indicates that the grains are $\sim 6.8 \pm 0.3$ nm tall by 46 ± 2 nm across, corresponding to ~ 20 unit cells laterally and vertically.

The coverage and thickness of the films on the SLG surface was evaluated by scanning electron microscopy (SEM). A top-down micrograph of a COF-5 film grown on SLG/Cu for 30 min (Fig. 2E) indicates complete coverage of the film over the graphene surface. A few bulk COF-5 crystallites, observed in greater frequency when longer reaction times are used, are scattered on top of the film. They are not strongly associated to the underlying film, and most are removed by sonicating the substrate in dry toluene for 10 s, after which the micrographs are uniform over $\sim 100\text{-}\mu\text{m}^2$ areas. Grain boundaries in the COF film appear in the micrograph as thin dark lines that we attribute to the roughness of the underlying polycrystalline Cu layer, as they are not

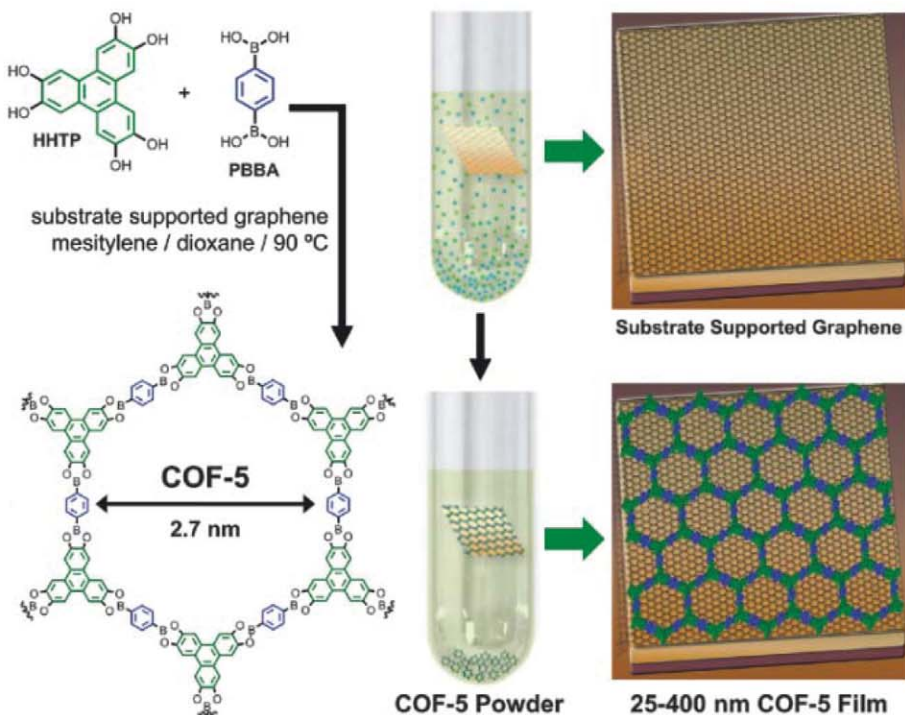


Fig. 1. Solvothermal condensation of HHTP and PBBA in the presence of a substrate-supported SLG surface provides COF-5 as both a film on the graphene surface, as well as a powder precipitated in the bottom of the reaction vessel.

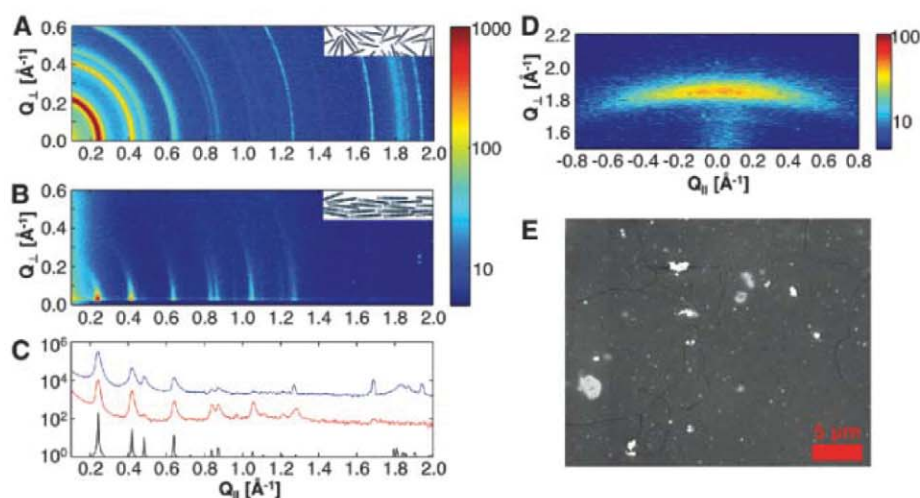
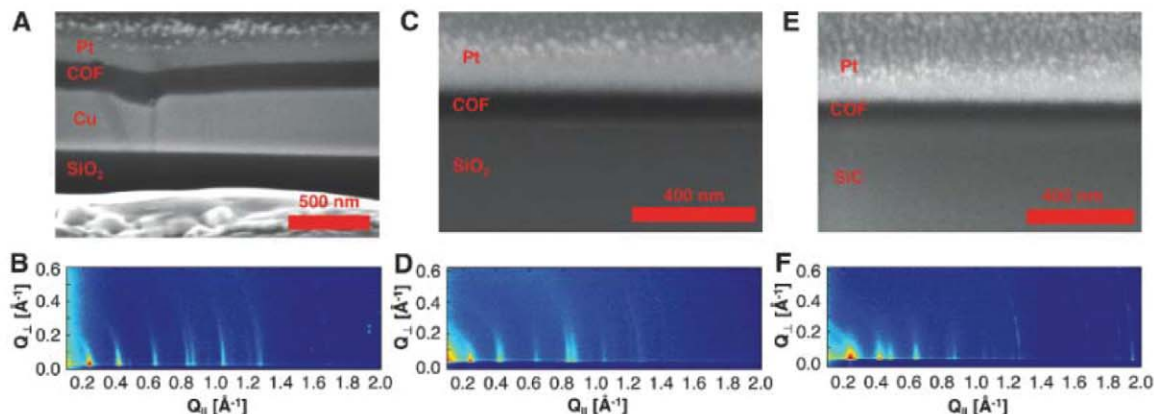


Fig. 2. (A) X-ray scattering data obtained from COF-5 powder; (inset) schematic of randomly oriented COF-5 grains in the powder, as indicated in (A). (B) GID data from a COF-5 film on SLG/Cu; (inset) schematic of oriented COF-5 grains in the film, as indicated in (B). (C) Projections of (A) (top/blue) and (B) (middle/red) near $Q_{\perp} = 0$, and the simulated powder diffraction spectrum (bottom/black) for COF-5. (D) GID data obtained at large $Q_{\perp}$, showing an off-specular projection of the COF-5 film (001) Bragg peak. (E) Top-down SEM image of the COF-5 thin film studied in (B), (C), and (D).

Fig. 3. (A) Cross-sectional SEM image of a COF-5 film on SLG/Cu (30-min growth time, 195 ± 20 -nm thickness) and (B) GID of the film. (C) Cross section of a COF-5 film on SLG/SiO₂ (2-hour growth time, 94 ± 5 -nm thickness) and (D) GID of the film. (E) Cross section of a COF-5 film on SLG/SiC (8-hour growth time, 73 ± 3 -nm thickness) and (F) GID of the film.



observed when COF-5 is grown on SLG on smoother substrates (for additional representative micrographs, see figs. S6 to S9). We obtained cross-sectional micrographs after depositing a protective layer of Pt (400 nm) and milling the sample with a Ga⁺ focused ion beam (FIB). The cross section of a film grown for 30 min (Fig. 3A) shows a continuous COF layer of 195 ± 20 -nm thickness, corresponding to ~ 580 layers. The GID of this sample (Fig. 3B) was identical to that obtained from the 2-hour film (Fig. 2B), indicating similar crystallinity and alignment. A discontinuity in the Cu is illustrated in Fig. 3A; though the structure of the graphene at this defect is not known, the COF film conforms to the indentation.

Although these studies were performed on SLG supported by its Cu growth metal, our synthetic method is general for SLG transferred to other substrates, including transparent fused SiO₂ (SLG/SiO₂). This flexibility facilitates studying the role of the underlying substrate on COF film growth and provides a direct route for incorporating COFs into a wide range of devices. COF-5 shows similar structure and alignment on SLG/SiO₂ compared with SLG/Cu. The GID of a film (Fig. 3D, 2 hours reaction time) exhibits the same 100, 110, 200, 210, 220, 310, 400, 320, 330, 420, and 500 Bragg peaks with diffraction intensities all localized near $Q_{\perp} = 0$. A cross-sectional micrograph (Fig. 3C) of the film obtained after FIB milling shows a COF-5 film thickness of 94 ± 5 nm, as well as a more uniform film/substrate interface compared with SLG/Cu. Top-down micrographs (fig. S10) show fewer bulk crystallites and none of the cracks observed in the films grown on SLG/Cu. Films grown on SLG/Cu are consistently thicker than those grown on SLG/SiO₂ at equivalent reaction times (fig. S11), suggesting that the Cu surface (including its defect sites) plays a role in COF nucleation. Because the graphene on each substrate is derived from the same CVD process, we conclude that the thickness and uniformity of the film is strongly affected by the quality of the underlying substrate.

COF-5 films also form on SLG derived from the thermal decomposition of SiC from its Si-terminated basal plane (SLG/SiC). SLG/SiC exhib-

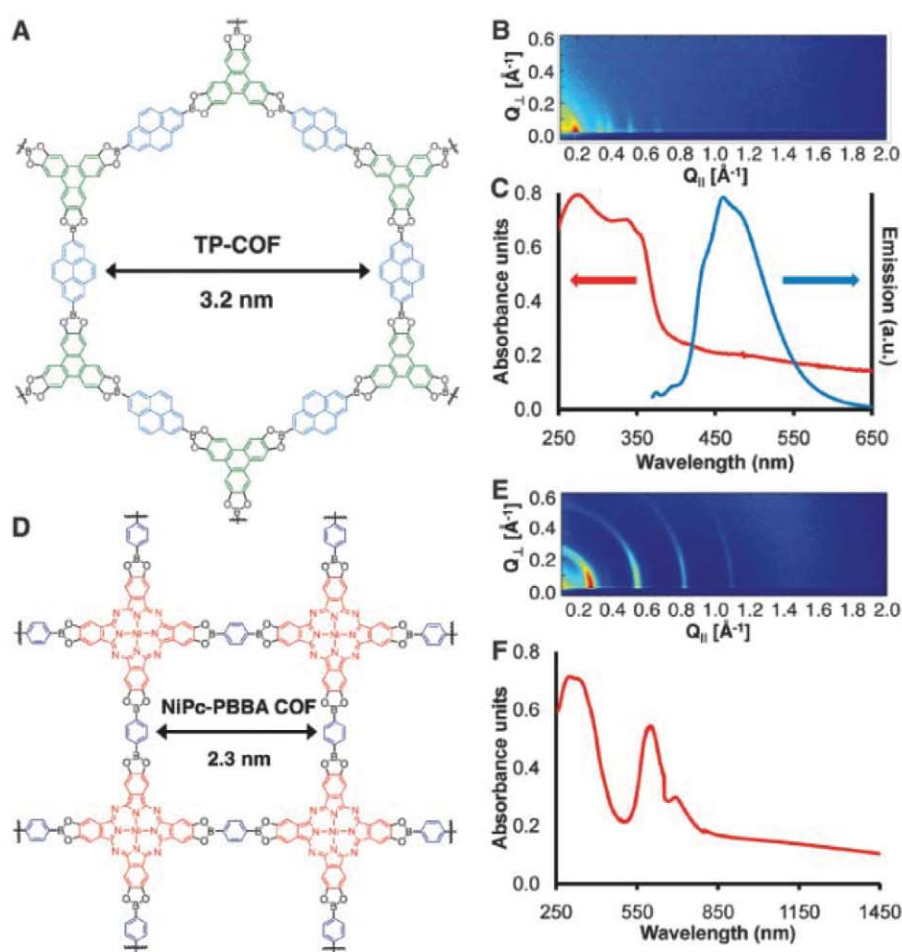


Fig. 4. (A) The TP-COF chemical structure, (B) GID of a film on SLG/SiO₂, and (C) transmission UV/Vis spectrum and emission spectrum ($\lambda_{\text{exc}} = 352$ nm) of the film. a.u., arbitrary units. (D) The NiPc-PBBA COF chemical structure, (E) GID of a film on SLG/SiO₂, and (F) transmission UV/Vis/NIR spectrum of the film.

its reduced surface roughness and larger graphene domains compared with SLG/Cu (26, 27). Top-down micrographs of COF-5 films grown for 8 hours indicate the formation of continuous films with no visible grain boundaries and few bulk crystallites (fig. S12). Cross-sectional micrographs obtained from FIB-milled samples indicate a uniform film with a thickness of 73 ± 3 nm (Fig. 3E and fig. S13). The relatively thin COF film

grown on SLG/SiC in 8 hours follows the thickness trend observed for SLG/Cu and SLG/SiO₂. GID of the film indicates similar diffraction patterns as those grown on the other substrates, suggesting a highly crystalline, vertically oriented film. The epitaxial relation between SLG and the single-crystal SiC substrate allowed us to determine that the COF-5 film does not grow epitaxially with respect to the graphene, as rotation of the sample

during the GID experiment did not reflect the sixfold symmetry of the COF lattice. This finding suggests that matching the COF lattice size and symmetry to the underlying graphene is not necessary to obtain crystalline films (see below).

The crystallinity and alignment of COF films on transparent SLG/SiO₂ substrates provides a means to organize functional π -electron systems within optoelectronic devices. Accordingly, films of two of the first COF semiconductors were grown on SLG/SiO₂. One of these frameworks, known as triphenylene-pyrene (TP)-COF (11), arises from incorporating a pyrene-2,7-diboronic acid linker in place of PBBA into the hexagonal COF-5 lattice (Fig. 4A). We obtained TP-COF in both thin-film and powder form using similar conditions to those described above (see figs. S14 and S15 for powder characterization and figs. S16 and S17 for top-down and cross-sectional micrographs). The GID of the films (Fig. 4B) indicates similar vertical alignment of the 2D lattice, as judged by the attenuation of the signals with increasing Q_z and the absence of the out-of-plane 001 diffraction. The increased pore size of TP-COF is apparent from the prominent 100 diffraction at $0.19 \text{ \AA}^{-1}$, and the 110 ($0.34 \text{ \AA}^{-1}$), 200 ($0.39 \text{ \AA}^{-1}$), and 210 ($0.52 \text{ \AA}^{-1}$) are also observed. Refinement of these data provided lattice parameters $a = b = 37.7 \text{ \AA}$ in excellent agreement with those derived from PXRD data of TP-COF powders ($37.5 \text{ \AA}$) (11). The transparent SLG/SiO₂ substrate enabled ultraviolet/visible/near infrared (UV/Vis/NIR) spectroscopy of a COF film in transmission mode for the first time (Fig. 4C, red trace). The spectrum is consistent with the presence of both HHTP and pyrene chromophores and shows improved vibrational resolution of the absorbance bands relative to the diffuse reflectance spectrum of the powder sample. The photoluminescence of the film (Fig. 4C, blue trace) is characteristic of pyrene excimer emission over all excitation wavelengths, arising from efficient en-

ergy transfer from HHTP to pyrene that was observed in TP-COF powders.

Finally, we confirmed that COFs lacking hexagonal symmetry may also be crystallized on SLG by preparing a Ni phthalocyanine-PBBA COF on SLG/SiO₂ (Fig. 4D). GID of the film (Fig. 4E) again exhibited diffraction peaks localized near $Q_z = 0$ located at $0.27 \text{ \AA}^{-1}$ (100), $0.55 \text{ \AA}^{-1}$ (200), $0.81 \text{ \AA}^{-1}$ (300), and $1.08 \text{ \AA}^{-1}$ (400). These data correspond to a vertically aligned 2D square lattice with parameters $a = b = 23.0 \text{ \AA}$ that match those obtained from the characterization of the powder sample. Cross-sectional images indicate a continuous film of $\sim 210 \pm 25$ -nm thickness (figs. S18 and S19). The translucent, turquoise films absorb strongly over the visible range of the spectrum as a consequence of the Ni phthalocyanine chromophores (Fig. 4F). Both the films and the powders are nonemissive, as is expected for H-aggregated phthalocyanines (see figs. S20 and S21 for powder characterization). These vertically aligned, porous phthalocyanine COFs are intriguing precursors of ordered heterojunction films long thought to be ideal for organic photovoltaic performance (28).

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A Virophage at the Origin of Large DNA Transposons

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DNA transposons are mobile genetic elements that have shaped the genomes of eukaryotes for millions of years, yet their origins remain obscure. We discovered a virophage that, on the basis of genetic homology, likely represents an evolutionary link between double-stranded DNA viruses and *Maverick/Polinton* eukaryotic DNA transposons. The Mavirus virophage parasitizes the giant *Cafeteria roenbergensis* virus and encodes 20 predicted proteins, including a retroviral integrase and a protein-primed DNA polymerase B. On the basis of our data, we conclude that *Maverick/Polinton* transposons may have originated from ancient relatives of Mavirus, and thereby influenced the evolution of eukaryotic genomes, although we cannot rule out alternative evolutionary scenarios.

Transposable elements (TEs) affect the genetic landscape of eukaryotes by creating new alleles, influencing gene regulation, and rearranging chromosome structure (1). TEs

can be divided into retrotransposons and DNA transposons. DNA transposons are further categorized into cut-and-paste TEs, rolling-circle TEs, and the self-synthesizing *Maverick* or *Polinton*

(MP) TEs (2). MP TEs are 9 to 22 kilobase pairs (kb) long, encode up to 20 proteins, and are found in a wide range of eukaryotes, including protists, fungi, and animals (3–6). Their coding capacity varies among species, yet they contain a conserved set of genes (4, 5). Most MP TEs encode a retroviral integrase, a viral protein-primed DNA polymerase B, an adenosine triphosphatase (ATPase) similar to the FtsK-HerA genome-packaging ATPases of double-stranded DNA (dsDNA) viruses, and a cysteine protease with homologs in adenoviruses. Another protein termed

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PY, which is frequently encountered in MP TEs (4), shows structural similarity to the major capsid protein of phycodnaviruses (7). Because MP TEs genetically resemble viruses of the bacteriophage PRD1-adenovirus lineage (8), a viral origin of these transposons has been proposed (5, 7), although a retroviral integrase in combination with the PRD1-adenoviral genes, as found in MP TEs, is not known in viruses.

The giant *Cafeteria roenbergensis* virus (CroV), with a 730-kb genome (9), replicates in the marine phagotrophic flagellate *C. roenbergensis*. We have discovered a much smaller virus associated with CroV infection (fig. S1) (10), which we named Mavirus (for Maverick virus). CroV production and host cell lysis were reduced by Mavirus infection (table S1), and Mavirus was not able to replicate in the absence of CroV, as demonstrated by quantitative polymerase chain reaction (qPCR) (table S2). We therefore classify Mavirus as a virophage sensu La Scola *et al.* (11), who coined the term “virophage” in reference to Sputnik, a true virus parasite of Mimivirus. According to this definition, virophages are predicted to have no nuclear phase in their infection cycle, to replicate in the virion factory of their host virus, and to be dependent on enzymes provided by their host virus, rather than by the host cell (11, 12). The discovery of Mavirus suggests that virophages may be a common phenomenon. The Mavirus genome (Fig. 1A) is a 19,063-base pair (bp) circular dsDNA molecule with a G+C content of 30.26% and 20 predicted protein-coding sequences (CDSs) of an average length of 883 nucleotides (nt), resulting in a genome-coding density of 92.6%. A nearly perfect inverted repeat of ~50 bp was situated in the intergenic region between CDSs MV20 and MV01. This region has the potential to adopt a hairpin structure with a 43-bp stem and a 15-nt loop (Fig. 1B). The adenine at the top of the loop was designated position 1 of the genome. Analysis of the intergenic regions revealed a conserved promoter motif (fig. S2) preceding all 20 CDSs that is highly similar to a putative late promoter motif in CroV (9). This implies that Mavirus gene expression is governed by the transcription machinery of CroV during the late stage of infection. Ten Mavirus CDSs showed sequence similarity to proteins from eukaryotes, bacteria, retroviruses, and dsDNA viruses, including at least four genes found in Sputnik (table S3 and fig. S3). Mavirus and Sputnik have homologous genes encoding a capsid protein (fig. S4), a predicted DNA-pumping ATPase (fig. S5), a predicted cysteine protease (fig. S6), and a predicted GIY-YIG endonuclease/Zn-ribbon protein (fig. S3); however, the integrases and promoter motifs (13) of the virophages are unrelated. Indeed, about 80% of the CDSs appear unrelated, suggesting that any evolutionary connection between these viruses must be ancient.

In contrast, a closer evolutionary relationship exists between Mavirus and MP TEs, which share seven homologous CDSs (Fig. 1A). One of the most conserved genes in MP TEs is the rve-

superfamily retroviral integrase (rve-INT; Pfam entry PF00665, 1e-16) (3–5), which contains a conserved CHROMO domain (PF00385, 5e-04) of ~60 amino acids capable of interacting with chromatin (14, 15). Usually, rve-INTs are expressed as part of the POL polyprotein, but in eukaryotes they can occur independently of retroelements in the form of Ginger DNA transposons (16) and cellular integrases (c-integrases) (15). MP TE-encoded c-integrases are the closest relatives to Mavirus protein MV02 (fig. S7). Another highly conserved gene found in MP TEs and Mavirus (MV03) is the predicted protein-primed DNA polymerase B (PolB) (fig. S8). For both rve-INT and PolB sequences, the closest identified relatives to Mavirus were found in a genomic contig of the slime mold *Polysphondylium pallidum*, which contains a MP-like element that is partially syntenic to Mavirus (Fig. 1C). This putative MP TE is truncated, and, consequently, no terminal-inverted repeats or target-site duplications were identified. A search for Mavirus-like promoter signals in *P. pallidum* was also unsuccessful. An additional Mavirus CDS with homologs in MP TEs is MV15, which bears Walker A, Walker B, and P9/A32-specific motifs that define the DNA-pumping ATPases of the FtsK-HerA clade found in membrane-containing

DNA viruses and MP TEs (fig. S5) (17). The adjacent CDS, MV16, encodes a predicted adenoviral cysteine protease with its closest homologs in Sputnik, MP TEs, and giant viruses (fig. S6). Homologs to three additional Mavirus genes—the superfamily 3 helicase, the major capsid protein, and MV19, which is similar to a cell wall surface anchor family protein from *Bdellovibrio bacteriovorus*—are occasionally encoded by MP TEs (4, 5, 7). Furthermore, both Mavirus and MP TEs have similar genome lengths (19 kb and 15 to 20 kb, respectively) and genome structures. MP TEs have terminal-inverted repeats of several hundred nucleotides in length and highly conserved ends that start with 5'-AG and end with CT-3' (4). Similarly, cutting the circular genome of Mavirus between nucleotides 19,063 and 1 (Fig. 1B) would result in a linear DNA molecule with 5'-AG and CT-3' termini, as well as 50-bp terminal-inverted repeats.

The observed parallels in genome length, genome structure, and gene content to MP TEs in general, as well as the sequence similarity and partial synteny to the MP-like element in *P. pallidum* in particular, imply that Mavirus and MP TEs have evolved from a common ancestor. The genetic similarities of these transposons and dsDNA viruses have spawned various evolution-

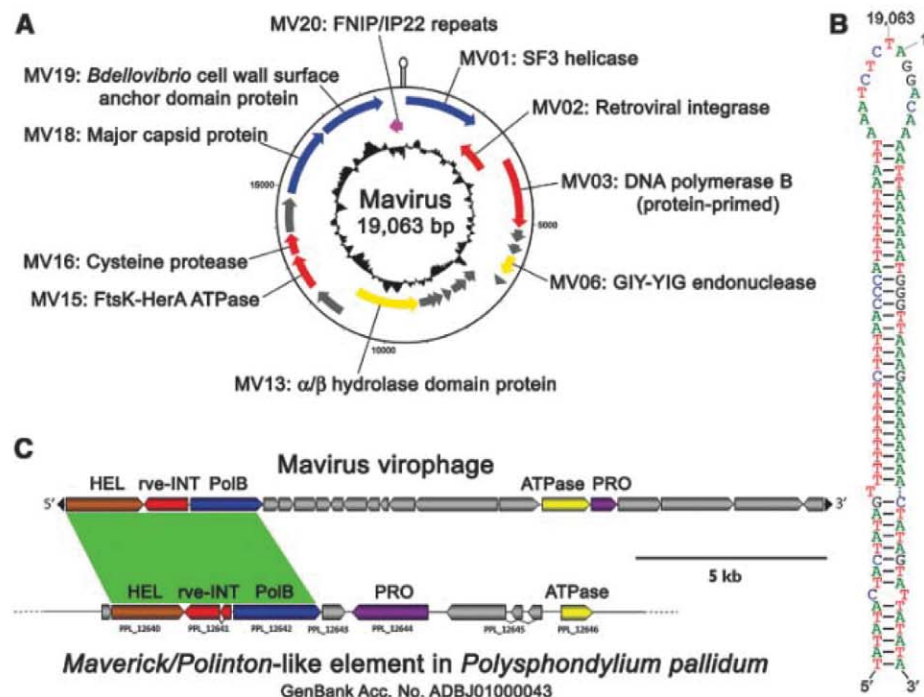


Fig. 1. (A) Genome diagram of the Mavirus virophage, indicating predicted protein functions. Circles from outermost to innermost represent protein-coding sequences (CDSs) on the forward strand, CDSs on the reverse strand, and G+C content (relative to the average of 30.26%). Conserved MP genes are shown in red; genes that are occasionally encoded by MP TEs are shown in blue. The FNIP/IP22 repeat-containing gene is shown in pink; additional genes with functional annotations are shaded yellow. A hairpin structure indicates the position of the inverted repeat upstream of CDS MV01. **(B)** DNA sequence and predicted secondary structure of the inverted repeat upstream of MV01. The first and last bases of the genome are numbered. **(C)** Gene organization of Mavirus (linear view of the circular genome) and of a truncated MP transposon in the slime mold *P. pallidum*. Homologous genes have the same color, and syntenic regions are shown in green.

ary hypotheses such as that MP TEs are derived from a linear plasmid (4), and that MP TEs and viruses of the PRD1-adenovirus lineage have evolved from a common ancestral virus (5, 7). Additionally, the origin of nucleocytoplasmic large DNA viruses has been linked to MP TEs (18). Although one can envision several scenarios that would explain the genetic homology between Mavirus and MP TEs, two likely candidates are that Mavirus was derived from an escaped TE, or that MP TEs arose from the endogenization of an ancient relative of Mavirus. The first scenario is problematic because it requires multiple gene-capture events by MP TEs from various sources. For example, the integrase would have to be acquired from a retroelement, whereas the cysteine protease and FtsK-HerA-like ATPase would have originated in a DNA virus. In addition, the first scenario fails to explain the genetic (figs. S3 to S6) and phenotypic similarities between Mavirus and Sputnik, as well as the dependency of Mavirus on CroV. A more parsimonious explanation is that a Mavirus ancestor (MVA) gave rise to MP TEs. This may have occurred in an early eukary-

ote susceptible to infection by a large DNA virus (LDNAV) that coevolved with the MVA (Fig. 2). MVA replicated by parasitizing the virion factory of LDNAV and interfered with its propagation, much as occurs in the CroV-Mavirus and Mimivirus-Sputnik systems. The interference of MVA with LDNAV replication led to increased survival of the host-cell population, and selection on the host cell to stabilize the relationship with MVA. In turn, the dependence of MVA on LDNAV replication provided strong selection for MVA to be closely associated with the LDNAV host. The fortuitous acquisition of an integrase gene by MVA provided a mechanism for integration into the cellular genome, which may have conferred protection against infection by LDNAV, and selective pressure for MVA to spread throughout the host-cell population. Selection for this viral defense system in early eukaryotes and repeated endogenization of various virophages in different eukaryotic lineages, combined with accumulation of mutations, intragenomic expansion, and partial or complete loss of the proviophage in some lineages, may have led to the current di-

versity and widespread, albeit patchy, taxonomic distribution of MP DNA transposons (Fig. 2) (4, 5).

In addition to their genetic similarities, the evolutionary relationship between Mavirus and MP TEs is supported by other observations. First, the integrases of Mavirus and MP TEs are closely related, suggesting that an ancestor of the Mavirus integrase could have catalyzed the insertion of DNA into a eukaryotic genome. We could, however, not detect endogenous forms of Mavirus in uninfected *C. roenbergensis* by PCR with Mavirus-specific primers. Second, Mavirus, as its ancestors presumably did, uses clathrin-mediated endocytosis to enter the host cell independently of its host virus, as shown by thin-section electron microscopy (fig. S1). This suggests that Mavirus can associate with the host cell even in the absence of a host virus. Third, Mavirus interferes with CroV propagation and increases the survival of the host-cell population (table S1); hence, eukaryotes that are susceptible to infection by giant viruses will gain a selective advantage if they can associate themselves with virophages. The directionality of the proposed viral transposogenesis is also supported by the shared promoter motif of Mavirus and CroV. It is hard to envision a mechanism that would have led to the de novo development from a transposon to the observed host-virus dependence, whereas it is conceivable that the promoter sequence degenerated after a MVA integrated into the host cell genome. In the light of recently described endogenous viruses (19), the discovery of Mavirus reveals a further facet of the intricate genetic interactions between viruses and cellular life.

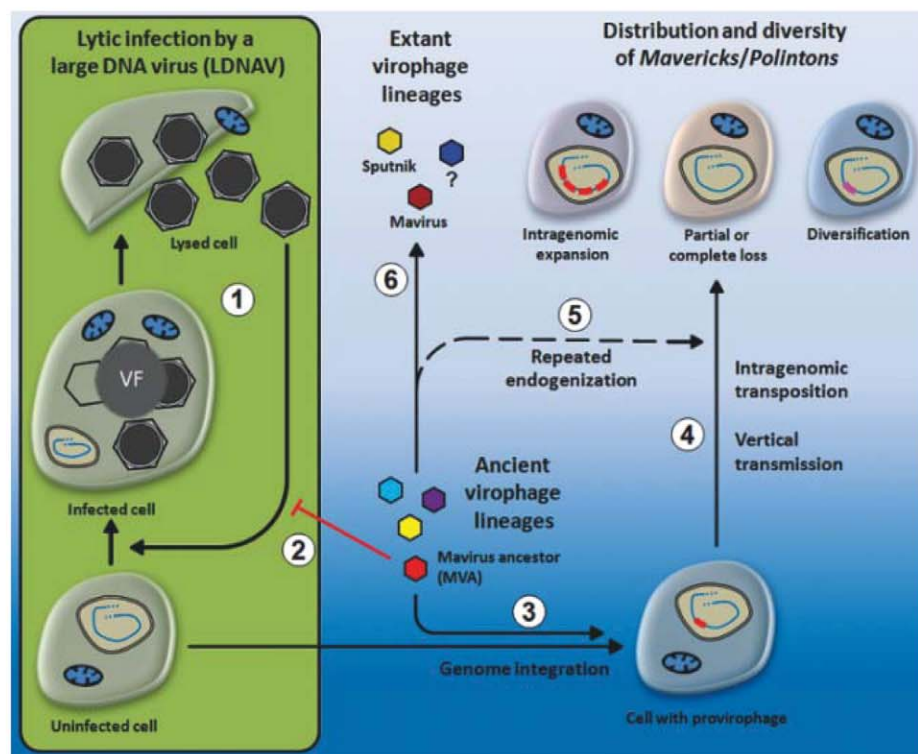


Fig. 2. Hypothesis for the origin of the *Maverick/Polinton* class of DNA transposons. (1) The infection cycle of a lytic large DNA virus (LDNAV). After virus entry, the virion factory (VF) produces progeny virions that are released during cell lysis. (2) A virophage (the Mavirus ancestor, MVA) is dependent on coinfection with the LDNAV and negatively affects its lytic infection cycle. The survival rate of the eukaryotic host population is increased by the MVA. (3) Using its integrase, the MVA inserts its genome into the host cell chromosome. (4) Over millions of years, the proviophage is vertically transmitted, accumulates mutations, expands horizontally in some eukaryotic lineages [e.g., *Trichomonas vaginalis* (5)], or suffers partial or complete loss in other lineages [e.g., Ascomycota (4)]. (5) These evolutionary processes are complemented by repeated endogenization of Mavirus-like virophages and result in the observed distribution and diversity of MP TEs. (6) Of presumably multiple ancient lineages of virophages, only the one from which Mavirus descended gave rise to the MP TEs. The Sputnik virophage has probably evolved from a different lineage, and many others may remain to be discovered.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1199412/DC1
Materials and Methods

Figs. S1 to S8
Tables S1 to S3
References

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A Dynamic Knockout Reveals That Conformational Fluctuations Influence the Chemical Step of Enzyme Catalysis

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Conformational dynamics play a key role in enzyme catalysis. Although protein motions have clear implications for ligand flux, a role for dynamics in the chemical step of enzyme catalysis has not been clearly established. We generated a mutant of *Escherichia coli* dihydrofolate reductase that abrogates millisecond-time-scale fluctuations in the enzyme active site without perturbing its structural and electrostatic preorganization. This dynamic knockout severely impairs hydride transfer. Thus, we have found a link between conformational fluctuations on the millisecond time scale and the chemical step of an enzymatic reaction, with broad implications for our understanding of enzyme mechanisms and for design of novel protein catalysts.

Protein motions are critical for biological functions, but their precise role in enzyme catalysis remains unclear (1, 2). Although there is convincing evidence that conformational fluctuations are essential for the mediation of substrate and cofactor binding as well as product release and can be rate-limiting for enzyme turnover (3–6), the importance of protein flexibility for progression along the chemical reaction coordinate remains a matter of debate (7, 8). In one view, electrostatic preorganization of the active site is considered to account fully for enzyme catalysis (9); however, hydrogen tunneling experiments suggest that a static model is inadequate and that fluctuations that reorganize the active site are required for the chemical step (7, 10, 11), which involves conformational sampling to facilitate hydrogen transfer as well as the transfer itself.

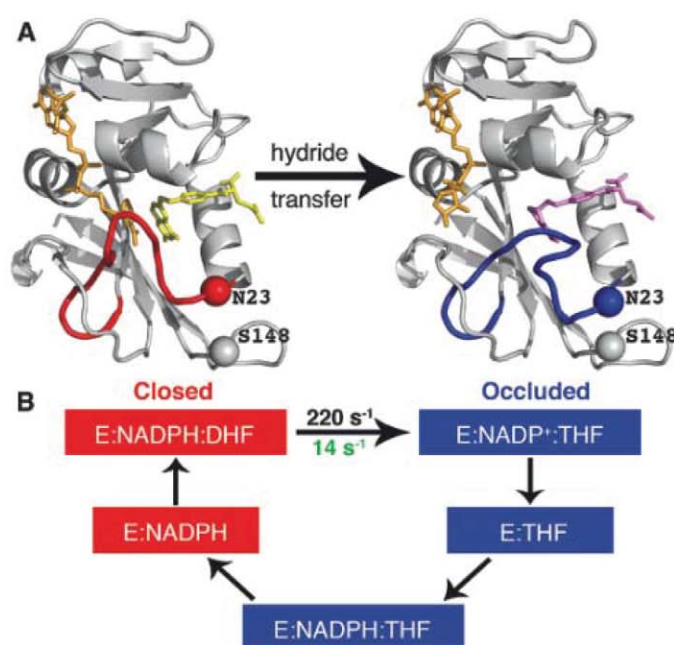
Escherichia coli dihydrofolate reductase (*ec*DHFR) is a paradigm for understanding the relationship between structure, dynamics, and catalysis (4, 12–14). DHFR catalyzes the stereospecific reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as cofactor. Five intermediates have been identified in the steady-state catalytic cycle (7): E:NADPH, E:NADPH:DHF, E:NADP⁺:THF, E:THF, and E:NADPH:THF (Fig. 1). Large conformational

changes are observed in the Met20 loop (residues 9 to 24) between the Michaelis complex E:NADPH:DHF [modeled by E:NADP⁺:folate (FOL) for structural studies] and the product

complex, E:NADP⁺:THF (13). The Met20 loop adopts two dominant conformations: the “closed” conformation, in which the loop packs tightly against the nicotinamide ring of the cofactor, and the “occluded” conformation, in which the loop projects into the active site and sterically blocks the nicotinamide-binding pocket. The holoenzyme (E:NADPH) and the model Michaelis complex (E:NADP⁺:FOL) are in the closed conformation, whereas the three product complexes (E:NADP⁺:THF, E:THF, and E:NADPH:THF) adopt the occluded conformation (Fig. 1), which is stabilized by hydrogen bonds between Asn23 and the backbone and side chain of Ser148 (13, 15).

In crystal structures of human and other vertebrate DHFRs, the Met20 loop is invariably closed (16). Alignment of the human and *E. coli* DHFR protein sequences suggests that the occluded conformation is destabilized in the human enzyme because of an inability to form the stabilizing hydrogen bond made by the S148 side chain in *ec*DHFR (fig. S1). In addition, a

Fig. 1. Conformational changes that occur during the *E. coli* DHFR catalytic cycle. (A) (Left) Illustration of E:NADP⁺:FOL crystal structure (1RX2, model for the Michaelis complex, E:NADPH:DHF) in the closed conformation. (Right) Crystal structure of E:NADP⁺:ddTHF (1RX4, model for the product complex) in the occluded conformation. NADP⁺ is shown in orange; FOL is shown in yellow, and ddTHF is shown in purple. Red indicates Met20 loop in the closed conformation; blue indicates Met20 loop in the occluded conformation. The sites of mutation, N23 and S148, are shown as spheres. (B) Intermediates in the wild-type *E. coli* DHFR catalytic cycle. Intermediates shown in red are in the closed conformation, and those in blue are in the occluded conformation. Before hydride transfer, the Met20 loop is in the closed conformation, in which it packs tightly against the nicotinamide ring of NADP⁺. After hydride transfer, the Met20 loop adopts the occluded conformation, in which the nicotinamide ring of NADP⁺ is sterically hindered from binding in the active site. NADP⁺ undergoes a concurrent conformational change in which the nicotinamide ring is expelled from the binding pocket, initiating NADP⁺ release from the ternary product complex. The rate of hydride transfer in the wild-type and N23PP/S148A mutant enzyme is indicated in black and green, respectively. The mutation alters the pathway used for product and NADP⁺ release, as shown in fig. S2.



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polyproline sequence (PWPP) at the end of the “Met20 loop” of human DHFR may render the loop less flexible than in the *E. coli* enzyme. We hypothesized that a “dynamic knockout” mutant designed to impair the dynamics of the Met20 loop in *ec*DHFR would give direct information on the role of active-site loop fluctuations in catalysis by the *E. coli* enzyme. To this end, the *ec*DHFR mutant N23PP/S148A (Fig. 1) was engineered and characterized by means of x-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and kinetic analysis (17, 18).

To assess the effect of the mutations on *ec*DHFR activity, we carried out both steady-state and pre-steady-state kinetic measurements on N23PP/S148A DHFR. The overall turnover rate (k_{cat}) is very much lower than for the wild-

type enzyme (Table 1). For wild-type *ec*DHFR, k_{cat} is determined by dissociation of the THF product from the E:NADPH:THF complex (Fig. 1) (10), whereas for the mutant enzyme the magnitude of k_{cat} is determined by dissociation of the NADP⁺ cofactor (fig. S2). Thus, the reduced flexibility inherent in the design of N23PP/S148A *ec*DHFR imposes a change in the kinetic step that determines the steady-state k_{cat} . The hydride transfer step was monitored directly in pre-steady-state kinetic experiments, as described in (17) (fig. S3). The rate of hydride transfer at pH 7.0 is 14 s^{-1} for the N23PP/S148A DHFR mutant, which is significantly slower than for wild-type *ec*DHFR (Table 1) (19). The measured rate for the chemical step has a primary kinetic isotope effect (KIE) of 3.0 (the same as the wild-type

enzyme), which is consistent with the difference in zero-point energies between the two hydrogen isotopes and confirms that we did indeed directly observe the rate of hydride transfer.

Structural differences between N23PP/S148A *ec*DHFR and wild-type *ec*DHFR were assessed by determining the crystal structure of the mutant enzyme in complex with NADP⁺ and folate and comparing it with the wild-type E:NADP⁺:FOL complex (13). To eliminate differences in refinement methods, the wild-type E:NADP⁺:FOL complex (13) was re-refined by using the structure factors deposited in the Protein Data Bank (PDB) (code:1RX2), as described in (17). The re-refined structure shows only very minor differences from the original 1RX2 coordinates. The N23PP/S148A *ec*DHFR at 1.6 Å resolution is almost identical to 1RX2–re-refined at 1.8 Å (Fig. 2) (13). Some minor adjustments at the end of the Met20 loop can be observed because of the proline insertion, but the loop is clearly in the closed conformation as in the wild-type E:NADP⁺:FOL complex. The ligand (fig. S4) and active-site side-chain conformations are maintained (Fig. 2), as are the hydrogen bonds (table S2) and the locations of water molecules in the active site. The distance between hydride donor and acceptor atoms is slightly decreased (from 3.3 to 2.9 Å) in the N23PP/S148A mutant. This decrease cannot account for the impaired hydride transfer rate in the mutant because a shorter distance would be expected to facilitate hydride transfer, not inhibit it. The similarity of the backbone conformations of the wild-type and N23PP/S148A E:NADP⁺:FOL complexes in solution is also illustrated by the similarity of ¹⁵N and ¹H chemical shifts (fig. S5).

The conservation of side-chain conformations and water molecules in the active site of the wild-type and mutant enzymes argues for a very similar electrostatic environment. However, a slight change in the Met20 side chain required further investigation. It has been proposed that the Met20 S_δ atom might assist catalysis by increasing the p*K*_a of the substrate (where *K*_a is the acid dissociation constant), promoting protonation of the N5 atom of the pterin (20). The Met20 S_δ atom in N23PP/S148A E:NADP⁺:FOL is shifted ~0.3 Å relative to its position in the wild-type complex (fig. S6) (21). We measured the pH-dependence of hydride transfer in the N23PP/S148A mutant to ensure that the apparent small difference in the position of the Met20 S_δ atom does not impair hydride transfer by decreasing the p*K*_a for N5. The hydride transfer rate for N23PP/S148A *ec*DHFR decreases with increasing pH (22) with a p*K*_a of 6.7 ± 0.1 , which is the same, within experimental error, as the value for the wild-type enzyme (6.5 ± 0.1). If anything, the p*K*_a is slightly higher for the mutant enzyme, which would facilitate hydride transfer rather than inhibit it. Thus, the x-ray and NMR data and the p*K*_a measurements show that there are no substantial differences in the structure or electrostatic environment

Table 1. Kinetic parameters. ND, not determined.

	Wild type*	N23PP-S148A	N23PP	S148A†
k_{cat} (s^{-1})	12	1.8 ± 0.6	2.5 ± 1	6.6 ± 0.8
k_{hyd} (s^{-1})	220 ± 10	13.9 ± 0.6	14.2 ± 1	157 ± 3
p <i>K</i> _a	6.5 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	ND
KIE (k_{hyd})	3.0 ± 0.1	3.0 ± 0.1	ND	2.7 ± 0.1

*Kinetic parameters for wild-type enzyme, as reported in (11).

†Kinetic parameters for the S148A mutant, as reported in (26).

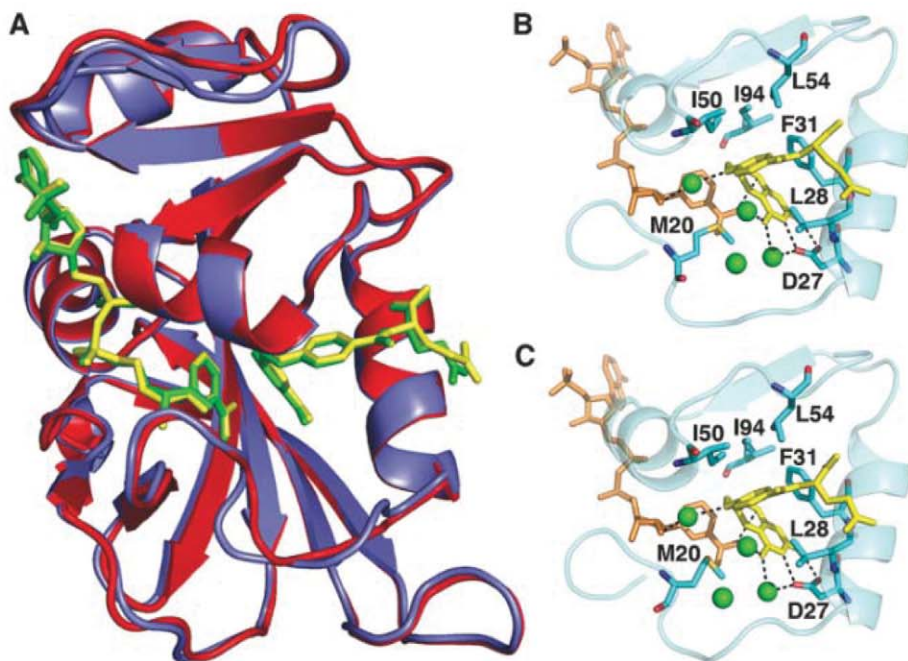


Fig. 2. The three-dimensional structures of the E:NADP⁺:FOL complexes of the N23PP/S148A mutant and wild-type *E. coli* DHFR are almost identical. (A) Superposition of the crystal structures of wild-type *E. coli* DHFR (1RX2) and N23PP/S148A *E. coli* DHFR. Wild-type *E. coli* DHFR is shown in red with yellow ligands, and N23PP/S148A *E. coli* DHFR is shown in purple with green ligands. (B) Active-site configuration for wild-type *ec*DHFR (1RX2–re-refined). Folate is shown in yellow, NADP⁺ is shown in orange, the waters are shown as green spheres, and key active-site residues are blue sticks. (C) Active-site configuration for N23PP/S148A *ec*DHFR. Colors are the same as for (B). For clarity, only the major conformation of the glutamate moiety of folate is shown. The active-site configurations are almost identical for wild-type and N23PP/S148A *ec*DHFR, including placement of polar residues, key hydrophobic residues, and waters, showing that the electrostatic nature of the active site is unchanged by the mutations.

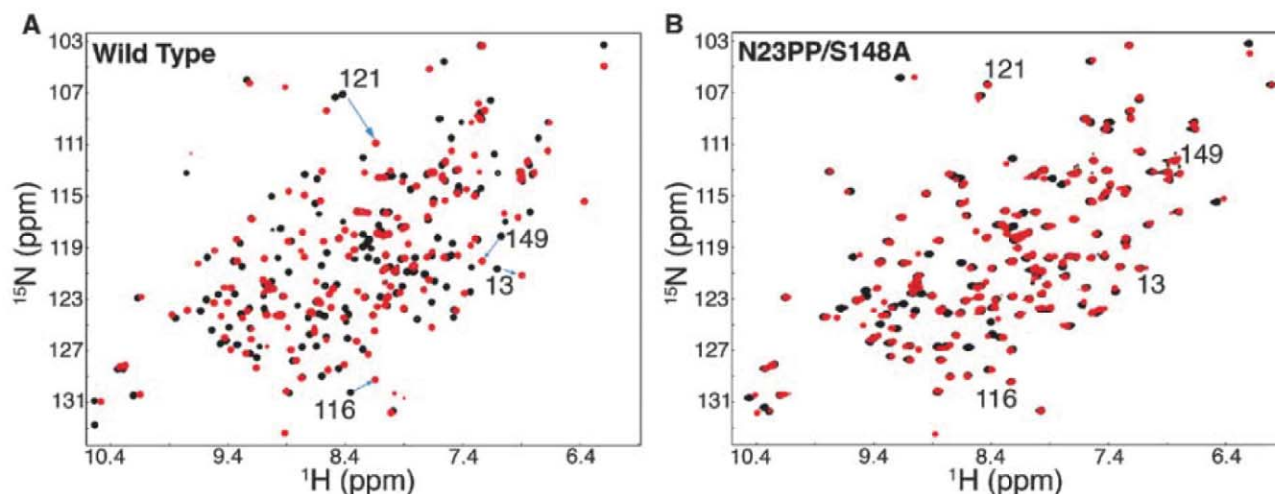


Fig. 3. The Met20 loop of N23PP/S148A *E. coli* DHFR remains in the closed position across the chemical step. **(A)** ^1H - ^{15}N HSQC spectra of wild-type E:NADP $^+$:FOL (model Michaelis complex, black) in the closed conformation and E:NADP $^+$:THF (product complex, red) in the occluded conformation. Large chemical shift differences are observed, particularly for residues in regions that undergo the closed-to-occluded structural change. Chemical shift changes from closed (black) to occluded (red) are indicated by arrows

for several residues in the active-site loops. **(B)** ^1H - ^{15}N HSQC spectra of N23PP/S148A E:NADP $^+$:FOL (model Michaelis complex, black) and E:NADP $^+$:THF (product complex, red). The ^1H - ^{15}N HSQC spectra for the complexes of the mutant enzyme are similar; the cross peaks corresponding to the active-site loops do not shift and appear in the position corresponding to the closed conformation for both complexes. A quantitative chemical shift analysis is presented in fig. S8.

of the active site between the E:NADP $^+$:FOL complexes of wild-type and N23PP/S148A *ec*DHFR.

In wild-type *ec*DHFR, the Met20 loop adopts the closed ground-state conformation in the Michaelis model complex, E:NADP $^+$:FOL, and the occluded ground-state conformation in the product ternary complex, E:NADP $^+$:THF (13, 15, 23). Previous NMR studies identified marker resonances, mostly in the Met20 and FG loops, for which ^{15}N and ^1H chemical shifts differ markedly between the closed and occluded conformations (23). Unlike the wild-type enzyme, the E:NADP $^+$:FOL and E:NADP $^+$:THF complexes of the N23PP/S148A mutant enzyme have almost identical ^1H and ^{15}N chemical shifts for most residues, including those in the Met20, FG, and GH loops (Fig. 3 and fig. S7), showing that the mutant enzyme remains in the closed conformation across the chemical step. The occluded conformation itself is not relevant to hydride transfer, which takes place within an ensemble of states in which the active site loops are closed (13). However, the fact that the mutant enzyme is unable to adopt an occluded conformation suggested that the flexibility of the active site loops may be considerably dampened and prompted us to examine the dynamics of the N23PP/S148A Michaelis model complex (E:NADP $^+$:FOL).

Carr-Purcell-Meiboom-Gill (CPMG)-based ^{15}N R_2 relaxation dispersion NMR experiments were used to monitor the sampling of higher-energy, lowly populated conformational substates that are associated with millisecond-time-scale structural fluctuations (24, 25). Previous studies of wild-type *ec*DHFR revealed millisecond-time-scale motions in the active site and C-terminal region in all five intermediate complexes of the catalytic cycle (4, 26). Surprisingly, relaxation dis-

persion experiments on the E:NADP $^+$:FOL complex of N23PP/S148A DHFR showed no dispersion for any active site residues (with the sole exception of Ala9), indicating that fluctuations on the millisecond time scale are abrogated in the mutant enzyme complex (Fig. 4). Residues associated with the C terminus, however, undergo fluctuations on the millisecond time scale similar to those in the wild-type enzyme, confirming that the mutations affect dynamics only in the active site. R_1 , R_2 , and heteronuclear nuclear Overhauser effect (NOE) experiments show that the Met20 loop of N23PP/S148A in the E:NADP $^+$:FOL complex is rather rigid on the picosecond to nanosecond time scale, as in the wild-type enzyme (fig. S8) (27). Several residues exhibiting exchange contributions from millisecond-time-scale conformational fluctuations in the active-site loops have elevated R_2 values in wild-type *ec*DHFR, whereas the values for these residues remain close to average for the N23PP/S148A mutant, suggesting that the mutations have not simply shifted the motions to a faster, microsecond time scale. No minor resonances, which might be expected if there was a small population (~3% or higher) of an alternative loop conformation in slow exchange with the closed ground state, were observed in the ^{15}N -HSQC spectrum of N23PP/S148A E:NADP $^+$:FOL. Thus, we conclude that the millisecond-time-scale fluctuations in the active site of the wild-type enzyme have been abolished in the N23PP/S148A mutant.

To obtain further insights into the effects of the N23PP/S148A mutations, we generated two additional *ec*DHFR mutants, N23PP and S148A. The N23PP mutation is in itself sufficient to abrogate the active-site flexibility required for efficient catalysis. The hydride transfer rate for

the N23PP mutant ($14 \pm 1 \text{ s}^{-1}$) is the same as for N23PP/S148A. NMR experiments show that the Met20 loop remains in the closed conformation across the hydride transfer step and that the millisecond-time-scale fluctuations in the active site are quenched in the E:NADP $^+$:FOL complex of N23PP DHFR (Fig. 4, Table 1, and fig. S9). An analysis of S148A *ec*DHFR reveals that the decreased hydride transfer rate does not simply arise from impairment of the closed-to-occluded transition in the mutant enzymes. S148A *ec*DHFR also remains in the closed ground-state conformation across the hydride transfer step (fig. S10), yet its hydride transfer rate (157 s^{-1}) is decreased only slightly from that of the wild-type enzyme (28). R_2 dispersion experiments on the S148A E:NADP $^+$:FOL complex show that although the millisecond-time-scale dynamics are dampened as compared with wild type, several active-site residues do retain flexibility, sampling higher-energy conformational substates that do not correspond to the occluded state (Fig. 4 and fig. S11). We conclude that unlike the N23PP and N23PP/S148A mutants, the S148A mutant retains residual motions in the active site, which are not associated with the closed-occluded transition but which probably play a role in promoting hydride transfer.

Considering that the E:NADP $^+$:FOL complexes, which are models for the Michaelis complex, have virtually identical active-site structures in both the N23PP/S148A mutant and wild-type enzyme, it is most unlikely that hydride transfer is impaired in the mutant because of differences in the structural and electrostatic environment of the active site. Although the ground-state structures are almost indistinguishable, the wild-type and mutant enzymes differ substantially in their ability to sample alternative ground-state active-site confor-

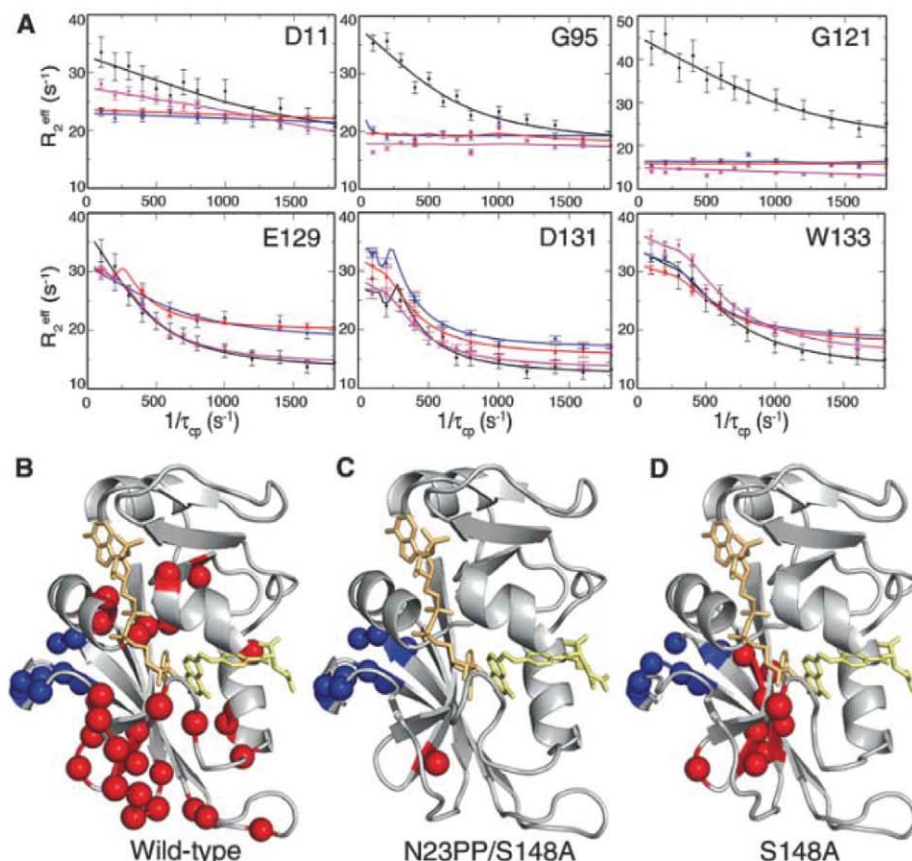


Fig. 4. Millisecond-time-scale dynamics in the active-site loops of the E:NADP⁺:FOL complex are impaired by the N23PP/S148A mutation. **(A)** Representative ¹⁵N R_2 relaxation dispersion curves for (black) wild-type, (red) N23PP/S148A, (blue) N23PP, and (purple) S148A E:NADP⁺:FOL. Dispersion data were collected at pH 7.6 and at 301 K. (Top) Active-site loop residues (11, 95, and 121), for which the dispersion observed in wild-type E:NADP⁺:FOL is not observed in either N23PP/S148A or N23PP E:NADP⁺:FOL. S148A retains dispersion for residue 11 and other active-site residues, as shown in fig. S11. (Bottom) C-terminal-associated residues (129, 131, and 133), for which dispersion is observed for the wild-type and mutant proteins. Residues that display ¹⁵N relaxation dispersion in the E:NADP⁺:FOL complex are mapped onto the structure for **(B)** wild type, **(C)** N23PP/S148A, and **(D)** S148A. 1RX2 coordinates are used for representations in **(B)** and **(D)**. Red spheres indicate active-site-associated residues for which R_2 dispersion is observed. Blue spheres indicate C-terminal-associated residues for which R_2 dispersion is observed. NADP⁺ is shown as orange sticks, and FOL is shown as yellow sticks. For clarity, only the major conformation of the glutamate moiety of folate is shown.

mations and access higher-energy conformational substates through millisecond-time-scale fluctuations. The ground-state conformational change between the closed Michaelis complex and the occluded product complex in wild-type *ec*DHFR is not observed for any of the three mutants because the active-site loops are restrained in the closed conformation. The millisecond-time-scale motions in the active site that allow the wild-type enzyme to sample higher-energy substates are eliminated in the N23PP/S148A and N32PP mutants, whereas the S148A mutant still retains some flexibility and has a correspondingly higher rate of hydride transfer. We therefore conclude that the greatly decreased rate of hydride transfer in the N23PP/S148A and N32PP mutant enzymes does not result from changes in structural or electrostatic preorganization of the active site but from impaired flexibility.

Molecular simulations consistently show that Met20 loop residues move closer to the cofactor on progressing from reactant to transition state. These residues are predicted to play an important role in electrostatic stabilization of the transition state and in promoting protonation of the N5 atom of the pterin moiety of the substrate (20, 29, 30). This movement is probably required to tightly close and compact the active site, to correctly orient and constrain the substrate and cofactor, and to promote hydride transfer by reducing the distance between the donor and acceptor atoms. Although the active site of the N23PP/S148A *ec*DHFR mutant is fully preorganized in the ground state, millisecond-time-scale fluctuations of the active site are restricted so that the enzyme cannot efficiently sample higher-energy conformational substates that are conducive to formation of the transition state; impairment of conformational fluctuations in the

active site leads to strong inhibition of the chemical step. Fine-tuning of conformational flexibility is probably a general phenomenon that can be harnessed in the engineering of efficient protein catalysts.

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18. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. In the mutants, other amino acids were substituted at certain locations; for example, H134R indicates that histidine at position 134 was replaced by arginine.
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22. At basic pHs, the hydride transfer rate (k_{hyd}) is decreased to 3.3 s^{-1} for N23PP/S148A because of the limited sources of protons for the hydride transfer step. The pK_a values are derived from fitting the pH versus k_{hyd} profile as described in (17).
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and analysis. G.B., P.E.W., and S.J.B. analyzed and interpreted the data. G.B., P.E.W., and J.L. wrote the manuscript. I.A.W., H.J.D., and S.J.B. edited the manuscript.

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Mutations in U4atac snRNA, a Component of the Minor Spliceosome, in the Developmental Disorder MOPD I

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Small nuclear RNAs (snRNAs) are essential factors in messenger RNA splicing. By means of homozygosity mapping and deep sequencing, we show that a gene encoding U4atac snRNA, a component of the minor U12-dependent spliceosome, is mutated in individuals with microcephalic osteodysplastic primordial dwarfism type I (MOPD I), a severe developmental disorder characterized by extreme intrauterine growth retardation and multiple organ abnormalities. Functional assays showed that mutations (30G>A, 51G>A, 55G>A, and 111G>A) associated with MOPD I cause defective U12-dependent splicing. Endogenous U12-dependent but not U2-dependent introns were found to be poorly spliced in MOPD I patient fibroblast cells. The introduction of wild-type U4atac snRNA into MOPD I cells enhanced U12-dependent splicing. These results illustrate the critical role of minor intron splicing in human development.

The small nuclear RNA (snRNA) U4atac is a component of the minor spliceosome and is required for the proper excision of the U12-dependent class of introns (1–4). Although they account for only about 800 introns in humans, U12-dependent introns are found in many essential genes, such as those involved in DNA replication and repair, transcription, and RNA processing and translation (5). Thus, mu-

tations in an snRNA required to splice such introns are likely deleterious and presumably would result in important developmental or clinical consequences.

U4atac snRNA appears to be encoded by a single gene (*RNU4ATAC*) on chromosome 2q14.2. Here we report that biallelic mutations in this gene are found in a severe developmental disorder, microcephalic osteodysplastic primordial dwarfism type I (MOPD I; also known as Taybi-Linder syndrome, OMIM 210710) (6). The main features of MOPD I patients are extreme intrauterine growth retardation, abnormalities in multiple organs, and death in infancy or early childhood (7, 8). We focused our initial studies on cases diagnosed in the Amish of Ohio (9) (fig. S1), where uniform phenotypic features and a high degree of consanguinity suggested the existence of a single founder mutation. Briefly, we applied genome-wide homozygosity mapping followed by targeted, high-throughput second-generation sequencing in search of mutations at chromosome 2q14.2 (Fig. 1 and figs. S2 to S4). A novel g.51G>A variant within the non-protein-coding *RNU4ATAC* gene was detected in homozygosity in all seven Amish patients studied and in

heterozygosity in 13 Amish parents. An Australian patient had the same mutation, whereas in two German MOPD I families, biallelic g.55G>A occurred in one patient and compound heterozygous g.30G>A and g.111G>A occurred in another patient. The 51G>A mutation represents a founder event in the Amish, as shown by haplotype analysis (figs. S3 and S5). This mutation was found in 16 of 281 Ohio Amish controls but in none of 180 Pennsylvania Amish controls. It was also seen in two of 720 controls from central Ohio but in none of 370 controls from France. The three mutations found in German MOPD I families were not found in 452 central Ohio controls. We conclude that the genetic findings are fully compatible with the expected recessive inheritance of rare mutations in the same gene [see (9) for further details of the mapping, sequencing, mutation analyses, and haplotyping].

The 30G>A, 51G>A, and 55G>A mutations in the U4atac snRNA are located within an important structural feature known as the 5' stem-loop; the 111G>A mutation is located in another essential stem region, the 3' stem-loop (10). These mutations are predicted to disrupt the snRNA's secondary structure and cause defects in the minor spliceosome (fig. S6) (11–13). To evaluate the functional effects of these mutations, we assayed the in vivo splicing of a modified U12-dependent intron reporter whose splicing is dependent on expression of a modified, exogenous U4atac snRNA, denoted U4atac-ATH (figs. S7 and S8) (10). Relative to wild-type U4atac, each of the MOPD I mutations in U4atac snRNA reduced U12-dependent splicing activity by greater than 90% (Fig. 2A). This suggests that all four mutations cause severe defects in U12-dependent splicing. Most of the splicing defect of the 51G>A mutation could be rescued by combining it with the 32C>T mutation in the presumed base-pairing partner (Fig. 2A, lane 8, and fig. S6). This suggests that the MOPD I mutation abrogates U4atac snRNA function by disrupting the RNA secondary structure.

U12-dependent introns are conserved in a variety of gene families implicated in different physiological processes (5, 14). To assess U12-dependent splicing in MOPD I cells, we compared fibroblasts obtained from two MOPD I patients (with the 51G>A mutation) and two normal human fibroblast cell lines. Using real-time reverse transcription polymerase chain re-

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action (RT-PCR) to quantify the levels of spliced and unspliced U12-dependent and U2-dependent introns, we found that all examined U12-dependent introns were less efficiently spliced in the MOPD I cells, whereas U2-dependent introns were not affected (Fig. 2B). Expression of wild-type U4atac snRNA in the MOPD I cells increased splicing of U12-dependent introns while having little effect on U2-dependent introns (Fig. 2C). Note that

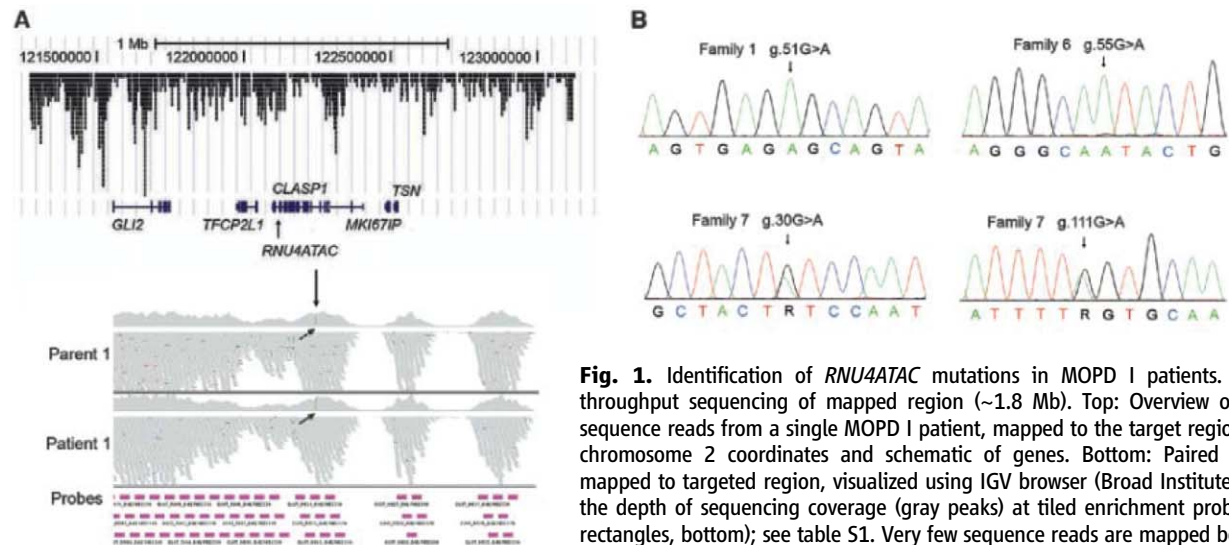
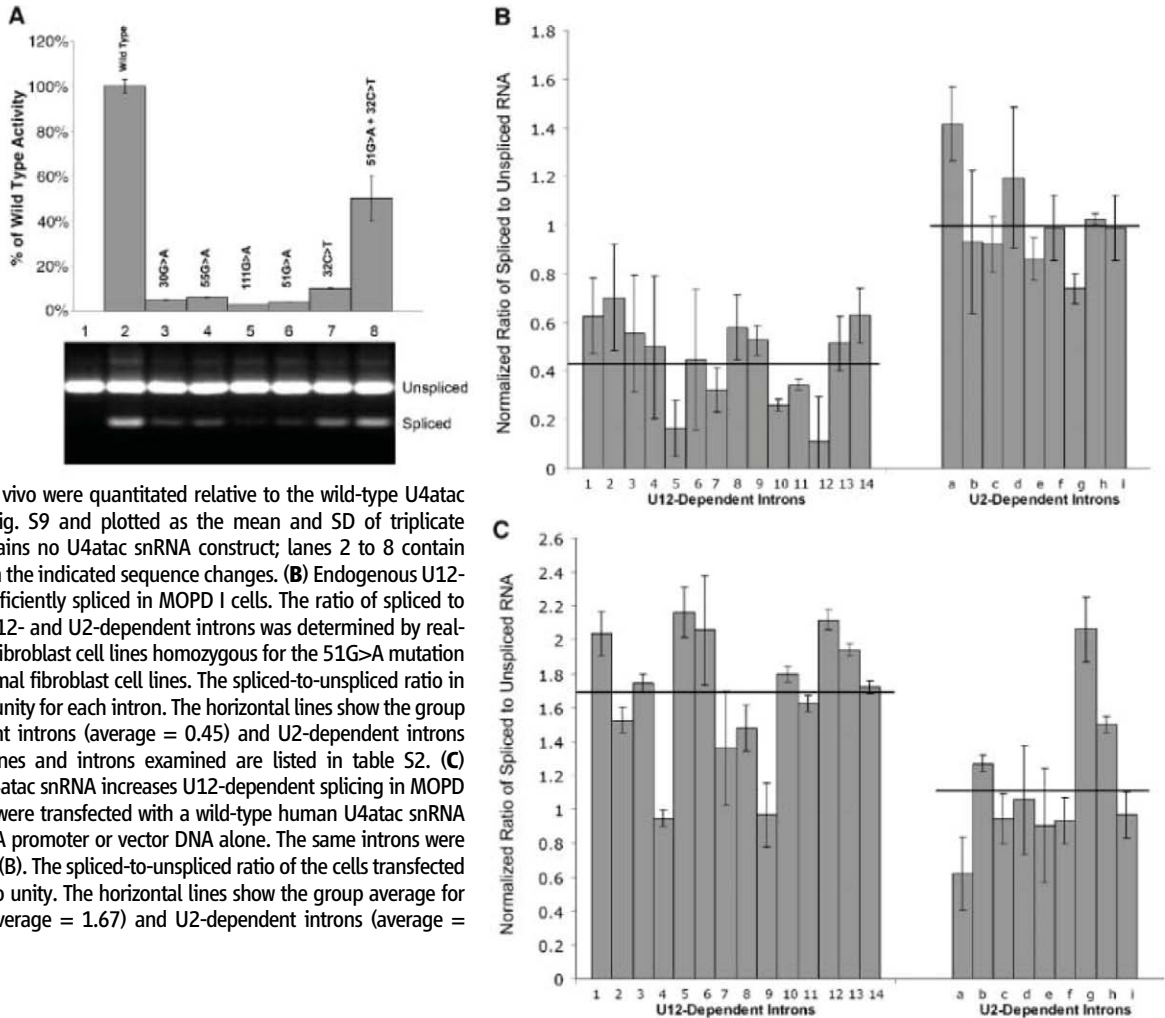


Fig. 1. Identification of *RNU4ATAC* mutations in MOPD I patients. **(A)** High-throughput sequencing of mapped region (~1.8 Mb). Top: Overview of enriched sequence reads from a single MOPD I patient, mapped to the target region showing chromosome 2 coordinates and schematic of genes. Bottom: Paired end reads mapped to targeted region, visualized using IGV browser (Broad Institute), showing the depth of sequencing coverage (gray peaks) at tiled enrichment probes (purple rectangles, bottom); see table S1. Very few sequence reads are mapped beyond 200 nucleotides into regions lacking enrichment probes. Dotted arrow: heterozygous mutation in parent 1; solid arrow: homozygous mutation in patient 1. **(B)** Conventional Sanger sequencing chromatograms show the distinct homozygous mutations (51G>A; 55G>A) and compound heterozygous mutations (30G>A and 111G>A) in MOPD I patients.

Fig. 2. Mutations in *RNU4ATAC* affect U12-dependent spliceosomal function. **(A)** U4atac snRNA mutations found in MOPD I patients disrupt minor class intron splicing in vivo. Chinese hamster ovary cells were transfected with a test intron and various snRNA constructs (fig. S8) (10). After 48 hours, RNA splicing products were analyzed by RT-PCR, followed by agarose gel electrophoresis to determine U12-dependent minor spliceosome activities. Effects on splicing in vivo were quantitated relative to the wild-type U4atac titration curve shown in fig. S9 and plotted as the mean and SD of triplicate transfections. Lane 1 contains no U4atac snRNA construct; lanes 2 to 8 contain U4atac-ATH constructs with the indicated sequence changes. **(B)** Endogenous U12-dependent introns are inefficiently spliced in MOPD I cells. The ratio of spliced to unspliced pre-mRNA for U12- and U2-dependent introns was determined by real-time RT-PCR. Two MOPD I fibroblast cell lines homozygous for the 51G>A mutation were compared to two normal fibroblast cell lines. The spliced-to-unspliced ratio in the normal cells was set to unity for each intron. The horizontal lines show the group average for U12-dependent introns (average = 0.45) and U2-dependent introns (average = 1.01). The genes and introns examined are listed in table S2. **(C)** Restoration of wild-type U4atac snRNA increases U12-dependent splicing in MOPD I cells. MOPD I fibroblasts were transfected with a wild-type human U4atac snRNA gene driven by a U1 snRNA promoter or vector DNA alone. The same introns were measured for splicing as in (B). The spliced-to-unspliced ratio of the cells transfected with vector alone was set to unity. The horizontal lines show the group average for U12-dependent introns (average = 1.67) and U2-dependent introns (average = 1.14).



introns 5 and 12, which are the most affected (Fig. 2B), also showed the greatest increase upon restoration of wild-type U4atac snRNA (Fig. 2C). We also observed considerable variability in the reduction of splicing of individual U12-dependent introns in patient cells (Fig. 2B). Such variability may underlie the specific developmental defects seen in MOPD I. Similar variability has been observed in *Drosophila* carrying a mutation in U6atac snRNA (15).

These results are fully compatible with the idea that the MOPD I mutations in U4atac snRNA reduce in vivo splicing of U12-dependent introns, with potentially deleterious consequences for proper levels of gene expression and/or alternative splicing. Our findings illustrate the critical role of the minor spliceosome in human development. Future work to define the downstream affected genes in MOPD I patients is warranted.

Several observations suggest that normal function of the minor spliceosome is crucial for viability and development (16, 17). Other human diseases caused by mutations in protein components of small nuclear ribonucleoprotein (snRNP) complexes that contribute to spliceosome functions have been described (18, 19). We found genetic mutations in the U4atac snRNA gene by high-throughput sequencing of the entire mapped genomic locus, notably including non-protein-coding DNA. Much of the current emphasis in finding the mutations underlying Mendelian disorders is focused on exome sequencing, which

would not have revealed the MOPD I mutations. This illustrates the need to sequence the genome rather than the exome in many situations.

Establishing a clinical diagnosis of MOPD (I, II, and III), Seckel syndrome, and related disorders has been difficult until now because of a considerable overlap in their clinical features (8, 20). In one study, mutations in the pericentrin (*PCNT*) gene were reported in patients with MOPD II but were not seen in patients with MOPD I or III, Seckel syndrome, or unclassified growth retardation (21). By contrast, other studies showed mutations in *PCNT* in patients with Seckel syndrome or MOPD II (22–24). Even among the cases reported here, there were clear differences in the phenotypic features and life spans. We anticipate that further characterization of distinct mutations in *RNU4ATAC* will shed light on some of these difficulties of clinical classification.

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Materials and Methods

SOM Text

Figs. S1 to S9

Tables S1 to S3

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Association of TALS Developmental Disorder with Defect in Minor Splicing Component *U4atac* snRNA

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The spliceosome, a ribonucleoprotein complex that includes proteins and small nuclear RNAs (snRNAs), catalyzes RNA splicing through intron excision and exon ligation to produce mature messenger RNAs, which, in turn serve as templates for protein translation. We identified four point mutations in the *U4atac* snRNA component of the minor spliceosome in patients with brain and bone malformations and unexplained postnatal death [microcephalic osteodysplastic primordial dwarfism type 1 (MOPD 1) or Taybi-Linder syndrome (TALS); Mendelian Inheritance in Man ID no. 210710]. Expression of a subgroup of genes, possibly linked to the disease phenotype, and minor intron splicing were affected in cell lines derived from TALS patients. Our findings demonstrate a crucial role of the minor spliceosome component *U4atac* snRNA in early human development and postnatal survival.

Taybi-Linder syndrome (TALS) is a rare autosomal recessive syndrome of as yet unknown etiology. Originally described in 1967 (1), a total of about 30 TALS cases have been reported (2). The TALS phenotype includes

marked intrauterine and postnatal growth retardation; short, bowed long bones with severe delay in epiphyseal maturation; severe microcephaly; brain malformations, including pachygyria or agyria; characteristic dysmorphic features; dry

skin; and sparse hair [supporting online material (SOM) text and fig. S1] and unexpected death within the first 3 years of life.

We originally mapped TALS to a 13-cM region (D2S2254 to D2S2215, 10 Mb) on chromosome 2q14 (3) in three consanguineous families (families F1 to F3, fig. S2) from the Mediterranean basin using a new homozygosity mapping approach. This approach relied on individual genome-based inbreeding estimates (4) rather than genealogical information, which is often limited. In the present study, we refined the TALS interval to 3.19 Mb by genotyping additional unaffected individuals from families F1 to F3 and a new consanguineous Moroccan family (family F4, fig. S2). No common haplotype appeared to be shared by TALS patients from families F1 to F4, ruling out a single founding effect.

Classical Sanger sequencing of candidate genes located within the newly refined 3.19-Mb TALS interval revealed no mutation (see SOM methods). This prompted us to perform targeted high-throughput sequencing of the region, rather than exome sequencing, in 10 selected individuals from TALS families F1 to F4. We selected one affected individual from each of the four TALS families and, to facilitate discrimination between rare variants and causative mutations by studying their segregation patterns, either both parents (families F2 and F4, fig. S2) or one healthy sibling (sib) not sharing the 2q14 haplotype of

the affected sib(s) (families F1 and F3, fig. S2). For each individual, more than 15 million 75-base high-quality reads were obtained, 75% of which were specific to the TALS 3.19-Mb genomic interval. 100% of the nonrepetitive 1.6-Mb region was captured at least once, with a mean sequencing depth of 300x. Mean sequencing depth was >30x for over 80% of this region. Among loci, 4356 bases differed from the reference DNA sequence, but only one substitution, a G→A change, segregated in TALS families F1 to F4 and met all criteria for a putative causative mutation; that

is, homozygous in probands, heterozygous in parents, and absent from healthy sibs not sharing the 2q14 haplotype of their affected sib(s) (table S1). The genomic 51 (g.51) G→A substitution is located within the *U4atac* small nuclear RNA (snRNA) gene, transcribed into a noncoding RNA that is unique to the minor spliceosome (5, 6). Sanger sequencing of all available individuals in TALS families F1 to F4 confirmed the presence of the g.51 G→A *U4atac* snRNA gene mutation. This mutation cosegregated with the disease and was concordant with linkage data (families F1 to F4, fig. S3).

Four additional TALS families (families F5 to F8) were then screened for *U4atac* snRNA mutations (fig. S3). Patient 8 (family F5) from India, without known consanguinity, was homozygous for the g.51 G→A *U4atac* snRNA gene mutation. Two unrelated white American non-consanguineous patients (patient 9, family F6; and patient 10, family F7) were compound heterozygotes, combining the g.51 G→A mutation with g.50 G→A or g.50 G→C mutation, respectively. Finally, compound heterozygosity for g.51 G→A and g.53 C→G mutations was found in a Norwegian nonconsanguineous patient (patient 11, family F8). Mutations cosegregated with the disease.

None of these *U4atac* snRNA gene mutations has been described as a polymorphism (dbSNP build 130 database, table S2). In addition, in an ethnically diverse control sample of 138 individuals, none of the three unique *U4atac* snRNA gene mutations was encountered, although one North African individual was heterozygous for the recurrent mutation. Thus, from this control population, disease incidence appears to be low. Estimation of TALS frequency as a function of ethnic origin will require sequencing of a larger control sample with diverse ethnic origins.

The *U4atac* snRNA is located within intron 2 of the *CLASP1* gene, −682 base pairs (bp) to −556 bp upstream of exon 3. Because of this, TALS mutations could, in theory, alter *CLASP1* splicing and/or expression. In silico splice site predictions, real time–quantitative polymerase chain reaction (RT-qPCR) analysis of *CLASP1* mRNA levels, and an RT-PCR study of *CLASP1* exon 3 splicing in TALS patients revealed no effects of TALS mutations on *CLASP1* expression or splicing (SOM methods and fig. S4).

U4atac forms a base-paired duplex with *U6atac*, required to activate minor splicing (Fig. 1). In the duplex, two intermolecular base-paired regions, stem I and stem II, are separated by an intramolecular base-paired 5' stem-loop (7). We investigated how the four TALS substitutions affect the structure of the *U4atac* snRNA molecule, and found that they disrupt the 5' stem-loop (fig. S5). In silico modeling of the *U4atac/U6atac* bimolecular structure predicts that these mutations drastically modify a site critical to 15.5-K protein binding and subsequent Prp31 protein binding (8–11). Binding of these proteins stabilizes the *U4atac/U6atac* U5 tri small nuclear ribonucleoprotein (snRNP) and is critical to splicing activity (12, 13). Accordingly, *U4atac* snRNA expression plasmids containing various point mutations or deletions within the 5' stem-loop were either inactive or showed partial splicing activity (10). It is therefore likely that TALS mutations within *U4atac* snRNA molecules affect minor splicing efficiency. Indeed, *U4atac* snRNA 50G, 51G, and 53C nucleotides are highly conserved across several species, including mouse, dog, and opossum (fig. S6). Several species such as zebrafish contain a g.51 G→A mutation, but in these cases there are compensatory mutations restoring the 5' stem-loop structure. The fact that the structure is tightly controlled across species

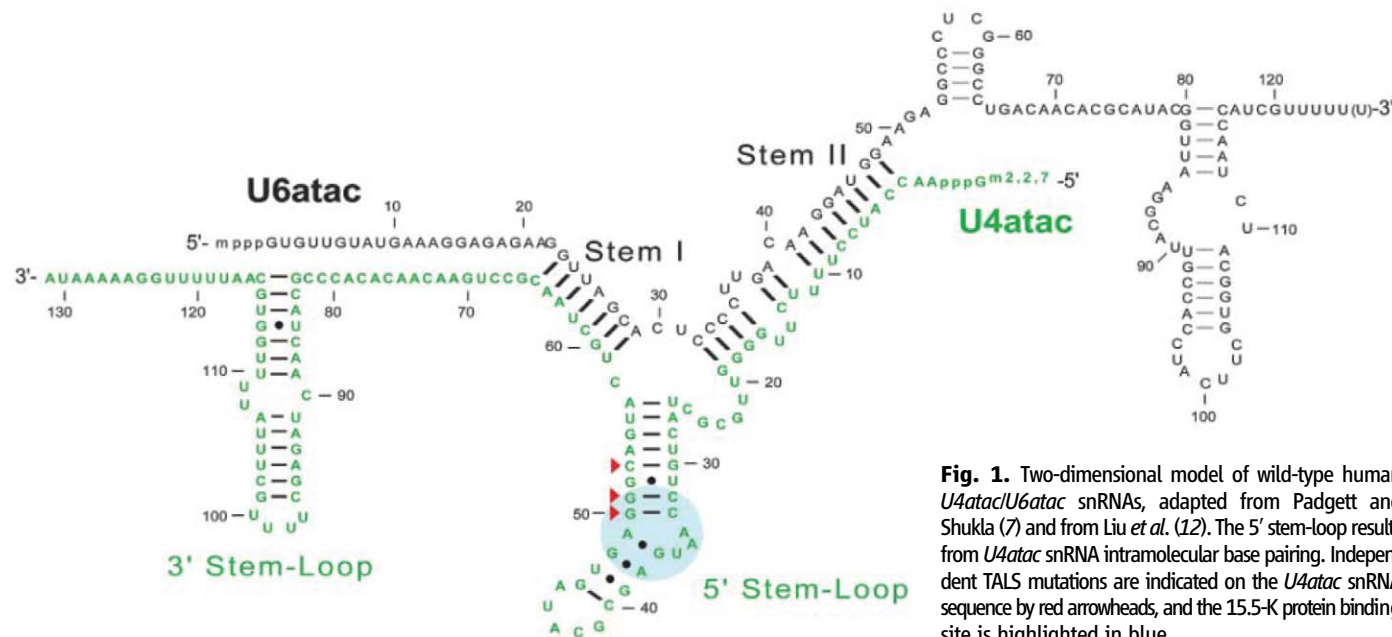


Fig. 1. Two-dimensional model of wild-type human *U4atac/U6atac* snRNAs, adapted from Padgett and Shukla (7) and from Liu *et al.* (12). The 5' stem-loop results from *U4atac* snRNA intramolecular base pairing. Independent TALS mutations are indicated on the *U4atac* snRNA sequence by red arrowheads, and the 15.5-K protein binding site is highlighted in blue.

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suggests that the sites mutated in TALS patients are structurally important and affect spliceosomal function.

The minor U12-dependent spliceosome is a ribonucleoprotein complex comprising *U11*, *U12*, *U4atac*, *U5*, and *U6atac* snRNAs. It is both structurally and functionally related to the *U1*, *U2*, *U4*, *U5*, and *U6* snRNAs of the major U2-dependent spliceosome; *U5* snRNA is shared by both spliceosomes. The human genome contains ~700 U12-type introns removed by the minor spliceosome (14, 15). U12-type introns are characterized by their consensus splice recognition sequences, combining a nearly invariant 5' splice site, either GTATCCT or ATATCCT, and a highly conserved branch site (16). Most genes containing U12-type introns (hereafter U12 genes) are either involved in key cellular functions such as DNA replication and repair, transcription, RNA processing and transport, translation, and cytoskeletal organization, or belong to a group of cellular ion channels. In TALS patients, *U4atac* snRNA gene mutations are expected to result in defects in minor splicing. This could produce abnormal transcripts containing either unspliced or alternatively spliced U12-dependent introns, often leading to the introduction of premature termination codons. In some cases, these aberrant mRNA molecules may be destroyed by nonsense-mediated mRNA decay as part of proofreading of nascent mRNA transcripts (17, 18). The effect of the homozygous recurrent g.51 G→A *U4atac* snRNA gene mutation on the expression of a subset of 23 genes spliced by the minor U12-dependent spliceosome was investigated. As expected in TALS fibroblasts, expression of several U12 genes was reduced (Fig. 2 and fig. S7). This could be the result of either rapid destruction of abnormal transcripts or down-regulated transcription. However, expression of a large group of U12 genes was normal or even slightly increased in TALS fibroblasts. In *Drosophila*, inactivation of *U6atac* snRNA led to mild perturbation of gene expression and splicing for most U12 genes, although the metabolic pathway downstream of mitochondrial prohibitin is particularly affected (19, 20). Expression of the human homolog of *Drosophila prohibitin*, the U12 gene *PHB2*, is also markedly reduced in TALS fibroblast cell lines (Fig. 2), suggesting that this gene or its downstream effectors may play a role in the TALS phenotype.

Splicing defects may lead to intron retention. We therefore used RT-qPCR experiments to examine the accumulation of transcripts retaining U12-type introns in TALS fibroblast cell lines. Statistically significant inhibition of minor intron splicing was detected in almost all U12-type introns tested (Fig. 3). Unspliced U12-type introns were increased up to sixfold in TALS versus control fibroblast cell lines, indicating a defect in minor spliceosome efficiency. Semiquantitative RT-PCR studies with gel visualization confirmed the defect in U12-type intron splicing in TALS fibroblast cells but showed the prevailing pres-

ence of normally spliced mRNA in these cells (fig. S8). Thus, the homozygous g.51 G→A *U4atac* snRNA recurrent mutation is "hypomorphic,"

reducing the activity of the minor U12-dependent spliceosome rather than completely abolishing it. Alternatively, an as yet unknown biological mech-

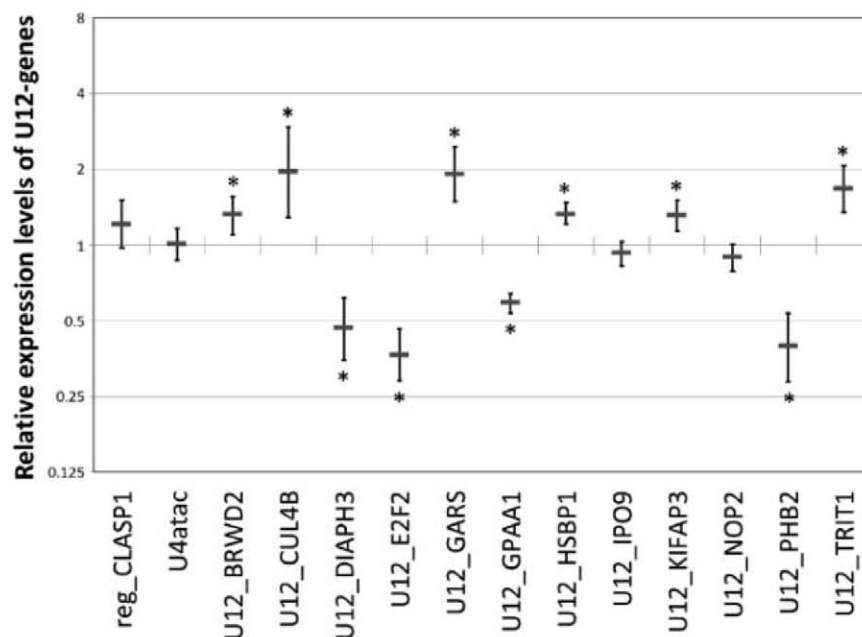


Fig. 2. A restricted number of U12 genes show decreased expression in fibroblasts derived from TALS patients. RT-qPCR determination of relative expression levels for U12 genes in fibroblasts derived from TALS2 and TALS6 patients, both harboring the g.51 G→A mutation, and fibroblasts derived from two control individuals matched for age and gender (six replicates for each experiment) are shown. Results within patient and control groups were similar; therefore expression levels are shown as mean values (12 replicates) with a 1 SD error bar (see SOM methods). Asterisks indicate statistical significance at 5%. The genes studied are indicated on the x axis: *CLASP1*, located within the refined TALS genomic interval; the *U4atac* snRNA gene; and 12 U12 genes. The y axis indicates relative expression ratios. mRNA expression was reduced for a subset of U12 genes (*DIAPH3*, *E2F2*, *GPAA1*, and *PHB2*) in TALS-derived fibroblasts. *U4atac* snRNA expression was normal.

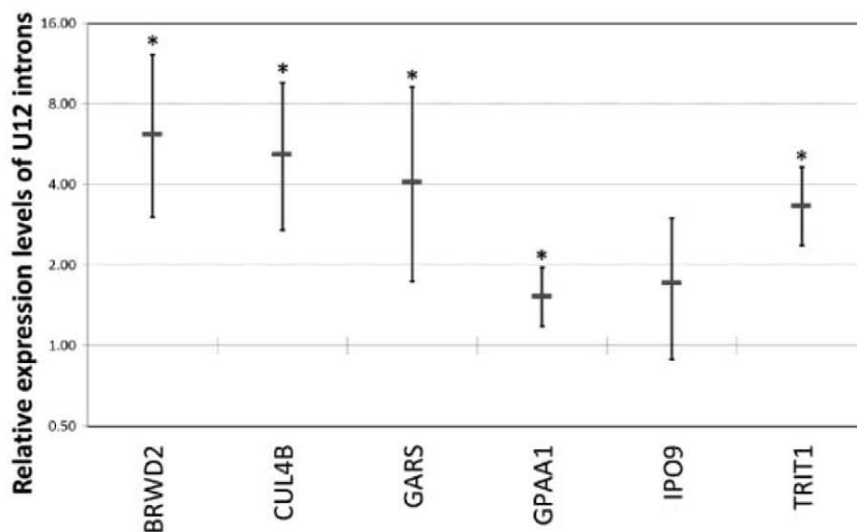


Fig. 3. Amounts of unspliced U12-type introns are markedly increased in mRNAs from TALS-derived fibroblasts. U12 introns from six U12 genes were quantified using RT-qPCR in TALS2- and TALS6-derived fibroblast cell lines. Results are expressed as relative values, as in Fig. 2. Asterisks indicate statistical significance at 5%. Unspliced U12-type introns detected in TALS-derived fibroblasts were up to six times more abundant than in control fibroblasts. For *IPO9*, only a slight increase in the unspliced U12-type intron was observed in TALS-derived fibroblasts. For *GPAA1*, the splicing defect for the U12 intron may be partially obscured by this gene's low expression level (Fig. 2).

anism may partly compensate for the minor spliceosome deficiency resulting from mutations in *U4atac* snRNA.

This paper shows compelling evidence that mutations in the *U4atac* snRNA gene are responsible for the early postnatal sudden death and severe brain and bone malformations seen in TALS, through minor spliceosome deficiency. Decreased expression of a restricted number of U12 genes and subsequent effects on downstream metabolic pathways may explain the TALS phenotype.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6026/240/DC1
Materials and Methods

SOM Text

Figs. S1 to S8

Tables S1 to S4

References

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Eosinophils Sustain Adipose Alternatively Activated Macrophages Associated with Glucose Homeostasis

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Eosinophils are associated with helminth immunity and allergy, often in conjunction with alternatively activated macrophages (AAMs). Adipose tissue AAMs are necessary to maintain glucose homeostasis and are induced by the cytokine interleukin-4 (IL-4). Here, we show that eosinophils are the major IL-4-expressing cells in white adipose tissues of mice, and, in their absence, AAMs are greatly attenuated. Eosinophils migrate into adipose tissue by an integrin-dependent process and reconstitute AAMs through an IL-4- or IL-13-dependent process. Mice fed a high-fat diet develop increased body fat, impaired glucose tolerance, and insulin resistance in the absence of eosinophils, and helminth-induced adipose tissue eosinophilia enhances glucose tolerance. Our results suggest that eosinophils play an unexpected role in metabolic homeostasis through maintenance of adipose AAMs.

Adipose tissue macrophages have a central role in promoting chronic low-grade inflammation, which contributes to obesity, insulin resistance, and type 2 diabetes, which characterize the metabolic syndrome (1). Although adipose macrophages from obese animals have a classically activated inflammatory phenotype, adipose macrophages from healthy lean mice have an alternatively activated phenotype (2). Impeding the ability of macrophages to become alternatively

activated by disrupting the nuclear hormone receptor peroxisome proliferator-activated receptor- γ (PPAR γ) renders mice susceptible to diet-induced obesity and glucose intolerance (3, 4). Human PPAR γ loss-of-function mutations are also associated with insulin resistance and type 2 diabetes (5). PPAR γ is induced in macrophages by interleukin-4 (IL-4) or IL-13 and promotes arginase-1 expression, one of the signature genes in alternatively activated macrophages (AAMs) (6). In vitro studies with adipocyte cell lines suggest that adipocytes themselves can be sources of IL-4 and IL-13 (7), but analysis of adipose tissues in vivo are needed to ascertain more definitively the source of these cytokines.

To begin an unbiased analysis of IL-4-expressing cells in perigonadal white adipose tissue of mice fed a normal commercial diet, we used IL-4 reporter mice (4get mice) (8), which contain a green fluorescent protein (GFP) reporter downstream of an internal ribosomal entry site element after the endogenous *Il4* gene, which facilitates recognition of IL-4-competent cells in vivo as revealed by their fluorescence (9). To

ensure minimal manipulation, we first analyzed cells spontaneously migrating out of minced adipose tissue after overnight incubation in medium. Although only small numbers of IL-4-expressing (GFP+) CD4+ T cells could be recovered, a large population of GFP+ eosinophils, which constitutively express GFP in 4get mice (9, 10), were identified (fig. S1A). We next enzymatically digested perigonadal adipose tissue to prepare a stromal vascular fraction (SVF) and recovered all IL-4-expressing cells for analysis. Of the IL-4-competent cells recovered from perigonadal adipose tissue of mice fed a normal diet, 90% were eosinophils, with the remainder made up of small numbers of basophils, CD4+ T cells, and innate helper type 2 cells (Fig. 1A, eosinophil gating in fig. S1B). Similar to the relatively abundant adipose tissue macrophages, adipose tissue eosinophils were CD11b+ F4/80+ but were distinguished both by GFP expression in 4get mice and by expression of the sialic acid-binding immunoglobulin receptor, Siglec-F (fig. S1, B and C). Analysis of adipose tissues from 4get mice with a *Gata1* promoter mutation that lack eosinophils (AdblGATA mice) (11) confirmed that the isolated cells were eosinophils (fig. S1D). Using mice with a knock-in human *CD2* replacement gene at the *il4* start site to mark cells that have recently secreted IL-4 protein (10), we could show that the majority of IL-4-secreting cells in adipose tissue were eosinophils, although the number of IL-4-secreting cells was only a small proportion of the total GFP+ IL-4-competent cells (fig. S2). Eosinophils up-regulate the inhibitory Siglec-F receptor as they move from blood into tissues (12). Compared with blood eosinophils, adipose tissue eosinophils show increased Siglec-F expression, consistent with tissue residence (fig. S1E). Eosinophils account for 4 to 5% of the adipose SVF cells, more abundant than total adipose CD4+ T cells (fig. S1F) or eosinophils in spleen ($0.33\% \pm 0.08\%$, mean % viable cells $\pm$ SEM) or blood ($2.4\% \pm 0.4\%$, mean $\pm$ SEM). Examination of perigonadal adipose tissue by

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standard histochemistry from wild-type (WT) mice identified eosinophils in normal perigonadal fat (Fig. 1B). Flow cytometric and immunohistochemical examination of adipose tissue from WT, eosinophil-deficient, and hypereosinophilic IL-5 transgenic (IL-5tg) mice (13) confirmed that Siglec-F-positive cells with the appearance of eosinophils were present in adipose tissue in numbers that correlated with the eosinophil status of the mice (Fig. 1, C and D). Eosinophils were also identified in perigonadal adipose tissue from WT C57BL/6 mice (Fig. 1, E and F). We noted a reciprocal relation between adipose tissue eosinophils and adiposity, as eosinophils were present but reduced in frequency in C57BL/6 mice fed a high-fat diet (Fig. 1, E and F) or in mice with genetic obesity secondary to leptin deficiency (*ob/ob*) (fig S3, A and B), and adipose tissue eosinophil

numbers correlated inversely with mouse weight (Fig. 1, G and H). Tissue eosinophils with up-regulated Siglec-F were present in perigonadal, subcutaneous, mesenteric, and brown adipose tissues; however, the frequency and absolute eosinophil numbers were highest in the metabolically active perigonadal and mesenteric adipose tissues (fig. S3, C and D), with the highest percentage of CD11b+ F4/80+ macrophages [perigonadal $32.4 \pm 3.5\%$; mesenteric $20.7 \pm 5.3\%$; brown $13.8 \pm 2.3\%$; and subcutaneous $1.2 \pm 0.08\%$ (mean $\pm$ SEM)].

To determine whether eosinophils migrate into adipose tissue, we transferred splenic eosinophils from hypereosinophilic mice (IL-5tg $\times$ 4get) to eosinophil-deficient mice. After 3 days, eosinophils were recovered from lung, spleen, and perigonadal fat. By 7 days, however, when eosinophils had declined substantially in lung

and spleen, eosinophils remained in stable numbers in adipose tissue (Fig. 2A) and in small intestine (fig. S4). Adoptively transferred eosinophils recovered from adipose tissue up-regulated Siglec-F, consistent with their tissue residence and distinct from the lower amounts of Siglec-F on spleen eosinophils harvested at the same time (fig. S5A). To confirm that eosinophil migration required transit from the blood to adipose tissue, we compared eosinophil accumulation in tissues of mice treated with antibodies that block vascular cell adhesion molecule-1 and intercellular cell adhesion molecule-1-mediated migration through α_4 and α_L integrins expressed on eosinophils (14). As compared with animals receiving isotype control antibodies, treatment with antibodies against integrin blocked accumulation of eosinophils in adipose tissues (Fig. 2B). Con-

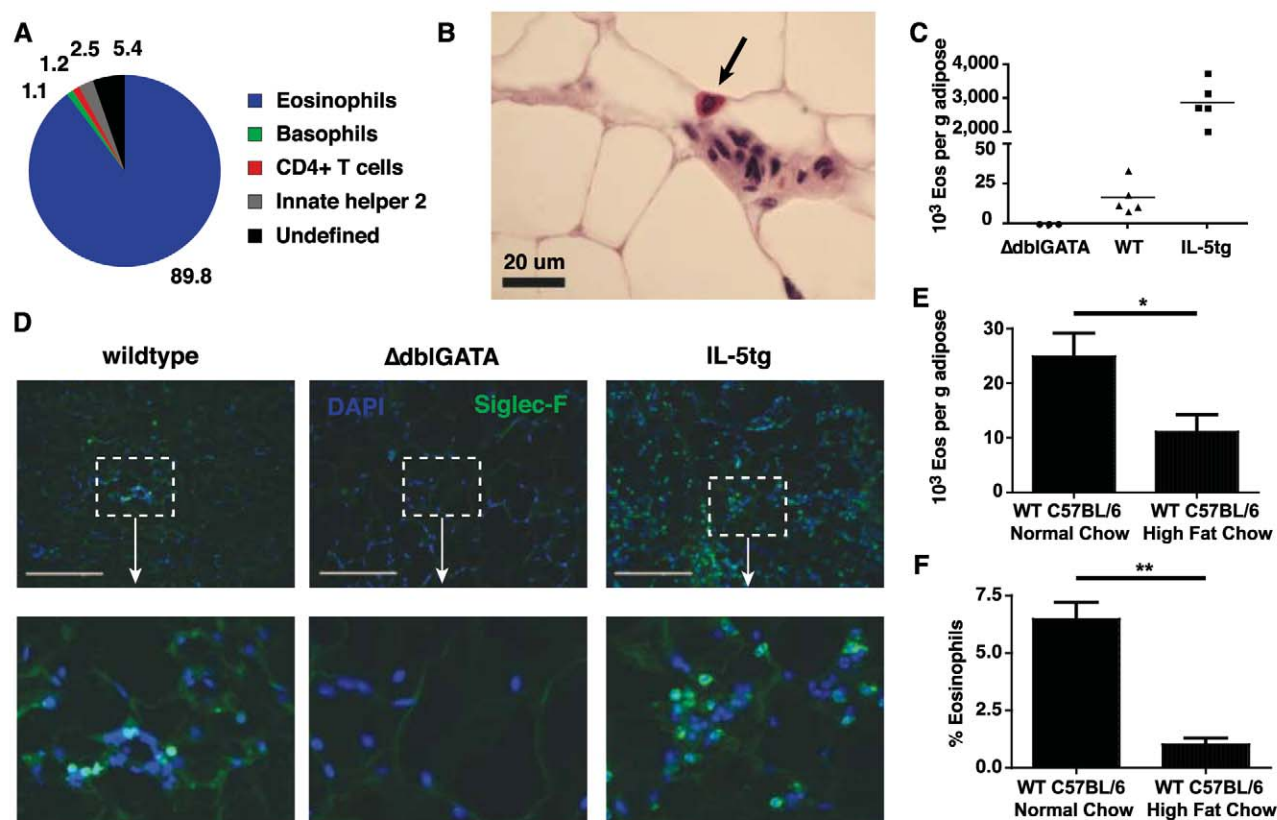


Fig. 1. IL-4-expressing cells in adipose tissue. (A) GFP-positive cells in perigonadal adipose tissue from 4get mice fed a normal commercial diet. Gating criteria are delineated in (8) and fig. S1B. Data were pooled from three independent experiments with two or more mice per group. (B) Hematoxylin-and-eosin stain of WT paraffin-embedded adipose tissue (scale bar, 20 μ m), representative of two independent experiments. (C) Eosinophil numbers as ascertained by flow cytometry in perigonadal adipose tissue from 8-week-old male mice from Δ dblGATA, WT, and IL-5tg mice fed a normal diet. Data are representative of three or more independent experiments. (D) Representative immunofluorescent images of Siglec-F+ cells in perigonadal adipose tissue from the strains indicated (scale bar, 50 μ m). Siglec-F, green; nuclei counterstain with 4',6'-diamidino-2-phenylindole (DAPI), blue; representative of two experiments. (E to H) WT male C57BL/6 mice were fed a high-fat diet (HF Chow) for 10 to 14 weeks and compared with WT C57BL/6 controls mice fed a normal diet (normal Chow). Perigonadal adipose

tissue eosinophils were quantified by flow cytometry (E) per g adipose tissue or (F) percentage of total SVF cells. Correlation is shown between the weight of mice fed a high-fat diet and their adipose tissue eosinophil numbers (G and H), by using Pearson's correlation coefficient. (E to H) Results are pooled data from two independent experiments with 20 total mice. * $P < 0.05$; ** $P < 0.01$.

versely, in lung and spleen, where most eosinophils retain low Siglec-F expression and presumably remain within the abundant vasculature of these

organs, eosinophils accumulated in animals treated with the integrin-specific antibodies. Integrin-specific antibodies similarly blocked eosinophil

migration into adipose tissue after induction of endogenous eosinophilia by IL-25 administration (15) (fig. S5, B and C).

Fig. 2. Eosinophil migration to adipose tissue is integrin-mediated. (A) Eosinophils in the left lobe of the lung, spleen, and perigonadal adipose tissue 3 and 7 days after adoptive transfer into eosinophil-deficient Δ dblGATA mice fed a normal diet. Data are pooled from two independent experiments. (B) Δ dblGATA mice received antibodies to α_4 and α_L integrins (100 μ g each) that block migration or control isotypes of immunoglobulin G (IgG2a and IgG2b) 2 hours before adoptive transfer of eosinophils. Tissues were harvested 16 hours later. Data are representative of two independent experiments. * $P < 0.05$; ** $P < 0.01$ as determined with Student's t test. n.s., not significant.

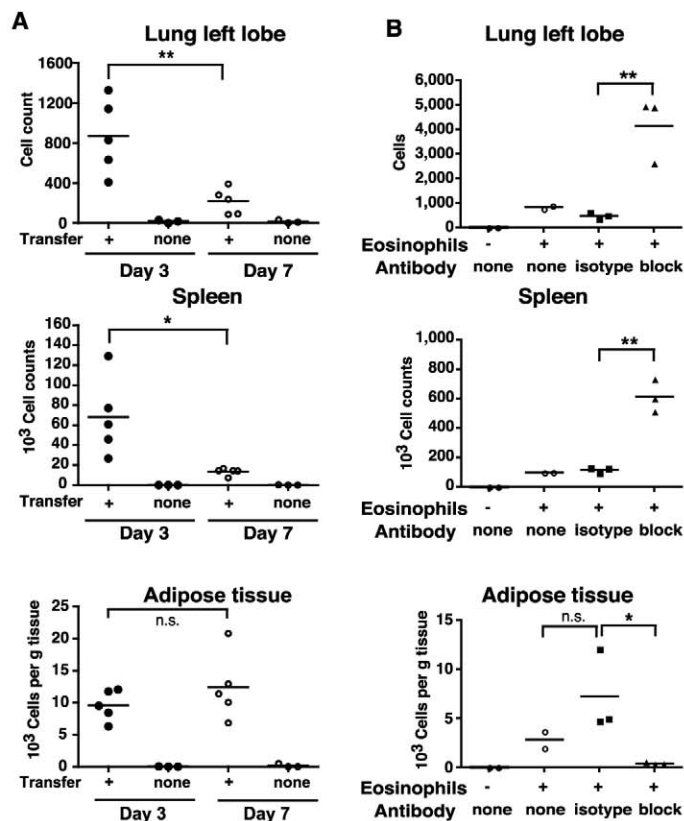
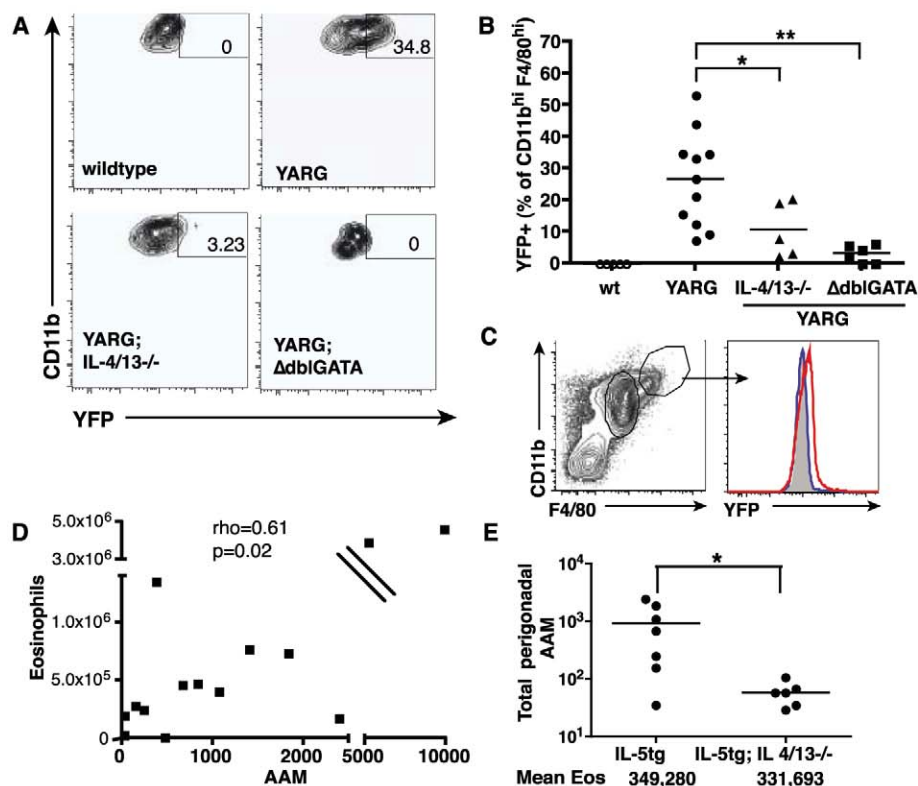


Fig. 3. Adipose macrophage alternative activation is impaired in the absence of IL-4 and IL-13 or eosinophils. (A) Flow cytometric analysis of adipose tissue from indicated mice fed a normal diet. Gates show YFP-positive cells as a percentage of total CD11b^{high} F4/80^{high} macrophages (A) and are quantified (B). Results are pooled data from two or more independent experiments with two to four animals per experiment. * $P < 0.05$; ** $P < 0.01$ as determined using analysis of variance (ANOVA) with Bonferroni's posttest correction for multiple comparisons. (C) Δ dblGATA $\times$ YARG mice were sublethally irradiated and reconstituted with bone marrow cells from 4get $\times$ IL-5tg mice. After 4 to 6 weeks, perigonadal adipose tissues were analyzed for eosinophils (left gate; eosinophils were GFP-positive and side-scatter^{high}, not shown) and macrophages (CD11b^{high} F4/80^{high}, right gate). Macrophages were then analyzed for YFP. Eosinophil-reconstituted mice (red); nonreconstituted mice (blue); WT control (non-reporter) mice (gray). (D) Statistical correlation (Spearman's rank correlation) between the total numbers of eosinophils reconstituting perigonadal adipose tissues in eosinophil-deficient mice and the total numbers of AAMs expressing the marker arginase-1 allele. Results are pooled data from five independent experiments with two to four animals per experiment. (E) Mice reconstituted with IL-5tg bone marrow lacking IL-4 and IL-13 (IL-5tg; IL 4/13^{-/-}) display significantly fewer total YARG⁺ AAMs (E) or total YARG⁺ AAMs per 1000 tissue eosinophils (IL-5tg 2.5 ± 1.1 ; IL-5tg $\times$ 4/13 DKO $2.4 \times 10^{-4} \pm 6.2 \times 10^{-5}$). Results are pooled data from two or more independent experiments with two to five animals per experiment. * $P < 0.05$; ** $P < 0.01$ as determined using Student's t test.



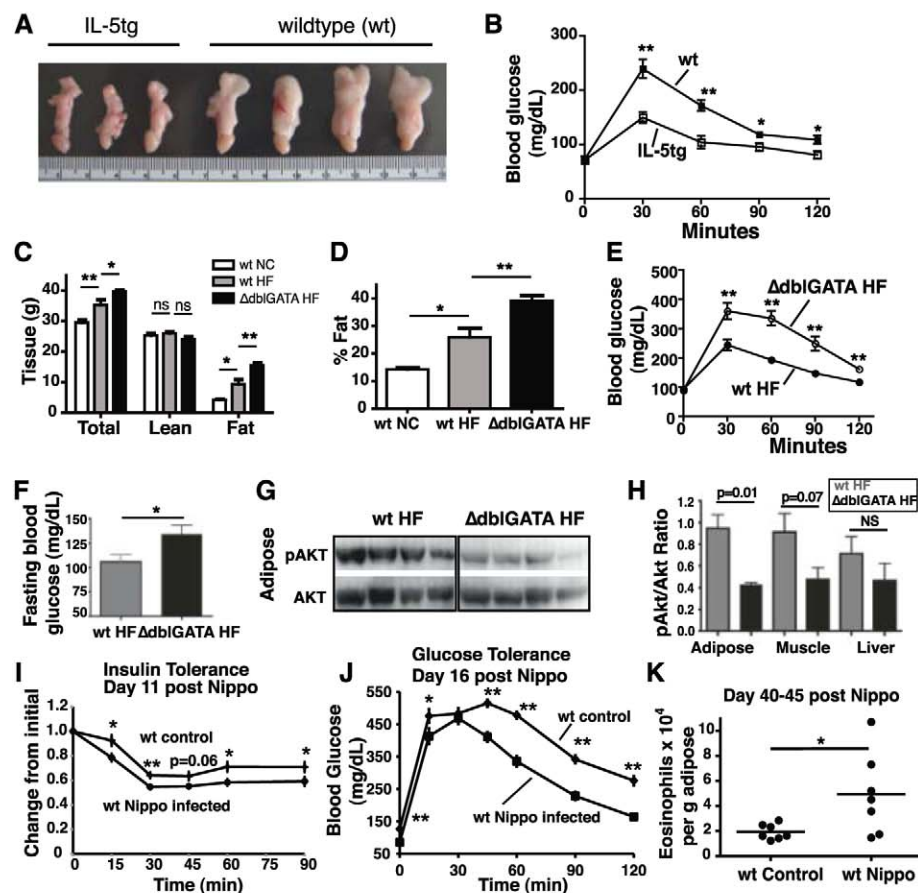
Studies of AAMs in adipose tissue have relied on polymerase chain reaction–based detection of signature genes in order to confirm the presence of these cells, but single-cell analysis has not been possible. Arginase-1 is a signature gene in mouse AAMs that is induced by IL-4 (6). We isolated cells in perigonadal fat from YARG mice, which have a fluorescent reporter introduced into the *arg-1* gene, that can be used to identify AAMs in vivo (16) (fig. S6). In both WT and YARG mice fed a normal diet, total numbers of CD11b⁺ F4/80⁺ macrophages were similar in adipose tissue (fig. S7), and in YARG mice, yellow fluorescent protein–positive (YFP⁺) macrophages were detected without further manipulation (Fig. 3A and fig. S7A). We used size-gating and Siglec-F expression to confirm that eosinophils do not express YFP fluorescence in YARG mice (fig. S7B). In the absence of IL-4 and IL-13 (17), numbers of total adipose macrophages remained similar (fig. S7C), but numbers of arginase-1–expressing macrophages diminished significantly (Fig. 3, A and B), consistent with a role for these cytokines in sustaining the phenotype of adipose AAMs under homeostatic conditions and in agreement with prior studies using Stat6-deficient mice (3). Contrary to our expectations, adipose tissues

from YARG mice that had been crossed onto the eosinophil-deficient background (YARG $\times$ Δ dblGATA) also demonstrated a significant reduction of AAMs in adipose tissue (Fig. 3, A and B), with similar numbers of total macrophages (fig. S7C). To ascertain whether eosinophils are sufficient to sustain AAMs in adipose tissue, we sublethally irradiated eosinophil-deficient YARG mice and reconstituted them with bone marrow cells from hypereosinophilic mice (IL-5tg $\times$ 4get). After 4 to 6 weeks, the perigonadal adipose tissues from reconstituted mice contained GFP⁺ eosinophils and YFP⁺ AAMs that were not present in nonreconstituted eosinophil-deficient mice (Fig. 3C). The numbers of eosinophils recovered within the perigonadal adipose tissue of the reconstituted mice correlated with the numbers of arginase-1–expressing macrophages in the same tissue (Fig. 3D). In contrast, animals reconstituted with IL-5tg bone marrow lacking IL-4 and IL-13 (double knockout) (IL-5tg $\times$ 4/13 DKO) had similar numbers of spleen and adipose tissue eosinophils, as well as similar adipose tissue weights, but did not restore AAMs (Fig. 3E and fig. S8, A and B). Thus, eosinophils and hematopoietic cell-derived IL-4–IL-13 are required to sustain adipose AAMs. Supporting these experiments, IL-4 and IL-13 transcripts

were found predominantly in the SVF rather than the adipocyte fraction of perigonadal white adipose tissue from WT mice (fig. S8, D and E).

During these experiments, it became apparent that the visceral adipose tissue of hypereosinophilic IL-5tg mice was visually smaller than the same tissues from littermate WT mice when both were fed a normal diet (Fig. 4A). As compared with WT mice, IL5tg mice had an improved response to a glucose challenge (Fig. 4B). Although differences in adipose tissue weight were not significant in eosinophil-deficient mice maintained on a normal diet, adiposity was greatly augmented when the mice were fed a high-fat diet. Under these conditions, analysis with dual-energy x-ray absorptiometry (DEXA) scans demonstrated significant increases in total body fat and percentage fat content in eosinophil-deficient mice as compared with WT littermates maintained on a high-fat diet, which suggested a role for eosinophils in protecting against diet-induced obesity (Fig. 4, C and D). The obesity induced in eosinophil-deficient mice fed a high-fat diet was metabolically relevant, because these mice demonstrated significantly impaired glucose tolerance compared with WT animals (Fig. 4E). When aged to 20 to 24 weeks while being maintained on a high-fat diet,

Fig. 4. Metabolic analysis of eosinophil-deficient and hypereosinophilic mice. (A) Perigonadal fat tissues (testis attached) from IL-5 transgenic (IL-5tg) and WT littermate controls. **(B)** Fasting male 8-week-old WT or IL-5tg littermates maintained on a normal commercial (NC) diet were challenged with intraperitoneal glucose, and blood was sampled for glucose at times indicated. Data were compiled from two independent experiments with six or seven mice in each group. **(C and D)** DEXA analysis of total, lean, and fat tissue composition (C) or percentage adiposity (D) in Δ dblGATA and WT mice fed a NC or high-fat (HF) diet for 15 weeks. Data were compiled from two experiments with five to eight mice in each group. **(E)** Intraperitoneal glucose tolerance test in male Δ dblGATA and WT mice fed HF diet for 15 weeks. Data were compiled from three independent experiments with five to eight mice in each group. **(F)** Fasting blood glucose in male WT and Δ dblGATA mice maintained on a HF diet for 20 to 22 weeks. Data were compiled from two independent experiments with five mice in each group. **(G and H)** Insulin signaling, as measured by the ratio of serine-phosphorylated Akt to total Akt in adipose tissue, muscle, and liver of mice aged 24 weeks fed a HF diet (n = four to six mice per genotype, four representative mouse adipose tissue samples shown). **(I and J)** Twelve-week old WT C57BL/6 mice fed a HF diet for 6 weeks were infected with *N. brasiliensis* (Nippo) or unchallenged (control) and monitored for (I) insulin tolerance and (J) glucose tolerance, at the indicated times. Insulin tolerance results are normalized to baseline fasting glucose, which was statistically different between cohorts (WT control 207 mg/dl $\pm$ 6; Nippo 179 mg/dl $\pm$ 7; P < 0.05). **(K)** Adipose tissue collected at days 40 to 45 post *N. brasiliensis* infection or from uninfected control mice and analyzed by flow cytometry for eosinophils per gram adipose tissue or percent eosinophils (WT control 2.9% $\pm$ 0.41; Nippo 10.1% $\pm$ 0.36, P < 0.01). Data (I, J, and K) are representative of



two independent experiments with 20 to 30 total mice per cohort. * P < 0.05; ** P < 0.01 as determined by Student's t test (B, E, and F, and H to K) or ANOVA with Bonferroni's posttest correction for multiple comparisons (C and D); error bars, SEM; n.s., not significant.

eosinophil-deficient mice had normal to mildly elevated levels of fasting insulin (fig. S9A), which indicated adequate pancreatic beta-cell insulin production. Eosinophil-deficient mice had elevated fasting blood glucose levels (Fig. 4F), however, and decreased adipose tissue insulin responsiveness, as shown by diminished tissue phosphorylated Akt levels after insulin challenge (Fig. 4, G and H).

To ask whether physiologic elevations of eosinophils could improve glucose tolerance in the setting of a high-fat diet, we infected mice fed a high-fat diet with a migratory helminth, *Nippostrongylus brasiliensis*. Although the parasite is cleared after 8 days, a sustained metabolic response was seen, characterized by decreased fasting glucose and improved insulin sensitivity and glucose tolerance present early post infection (Fig. 4, I and J) and sustained up to 35 days post infection (fig. S9B). Remarkably, decreased perigonadal adipose tissue weight (control $2.4 \text{ g} \pm 0.12$; infected $1.8 \text{ g} \pm 0.15$, $P < 0.01$) and increased perigonadal adipose tissue eosinophils were also maintained up to 45 days post infection (Fig. 4K), when spleen, liver, and bone marrow eosinophils had returned to baseline (fig. S9C). Total adipose macrophages from the infected cohort were also relatively decreased (control $50.6\% \pm 2.7$; infected $35.3\% \pm 2.0$, $P < 0.05$), consistent with a decline of classically activated macrophages known to preferentially populate adipose tissue during high-fat feeding (2).

Despite much literature defining a role for AAMs in sustaining insulin sensitivity and glucose homeostasis, the mechanisms responsible for maintaining these cells in healthy adipose tissues are largely unknown. Although differences exist between mouse and human AAMs, including in the expression of arginase-1 as used here, we note the overlapping profiles of IL-4- and IL-13-conditioned human monocyte/macrophages that have also allowed recognition of human AAMs (6). Our work suggests that eosinophil IL-4 production may contribute to sustaining AAMs; however, additional contributions from eosinophils are likely important, possibly through production of cytokines, chemokines, or other mediators. Furthermore, we note that innate helper 2 (ih2) cells, recently implicated as an important source of IL-5 and IL-13, are present in adipose tissues (Fig. 1A), as previously noted (18), and future study will be required to address whether cytokines from these cells maintain adipose tissue eosinophil homeostasis. Additional contributions by adaptive immune cells likely add further complexity and fine control (19–21).

Despite their appearance in allergy and states of parasitism, particularly in response to intestinal helminthes, the biologic role of eosinophils remains incompletely defined. Although sparse in blood of persons in developed countries, eosinophils are often elevated in individuals in rural developing countries where intestinal parasitism is prevalent and metabolic syndrome rare (22). We speculate that eosinophils may have evolved to optimize metabolic homeostasis during chron-

ic infections by ubiquitous intestinal parasites (23), and in contrast to the insulin-resistant state induced by acute microbial infections (24). Our findings further support the intertwined relationship between metabolism and immunity (1) and are consistent with the enrichment of inflammatory and immune response genes associated with obesity in humans (25, 26). Modulating adipose tissue eosinophil number and function could provide an exciting and novel therapeutic target in human metabolic disorders.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S9

References

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AMP-Activated Protein Kinase Regulates Neuronal Polarization by Interfering with PI 3-Kinase Localization

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Axon-dendrite polarization is crucial for neural network wiring and information processing in the brain. Polarization begins with the transformation of a single neurite into an axon and its subsequent rapid extension, which requires coordination of cellular energy status to allow for transport of building materials to support axon growth. We found that activation of the energy-sensing adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) pathway suppressed axon initiation and neuronal polarization. Phosphorylation of the kinesin light chain of the Kif5 motor protein by AMPK disrupted the association of the motor with phosphatidylinositol 3-kinase (PI3K), preventing PI3K targeting to the axonal tip and inhibiting polarization and axon growth.

Morphological polarization sets the foundation for synapse formation and efficient information transfer in the brain. Polarization is initiated by the selective rapid growth of a single neurite that will eventually differentiate into an axon, whereas the remaining sister neurites become dendrites. The scale of axon growth during neuronal polarization requires both a large energy supply to support increased protein and membrane synthesis and extremely active intracellular delivery (1). Cellular energy status could thus be a key factor in initiating axon outgrowth and polarity formation.

In all cell types, including neurons, the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) signaling cascade measures bio-

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energy homeostasis, with its activity level inversely related to energy sufficiency (2), and is implicated in brain development (3).

To investigate the role of AMPK activity in neuronal polarization, we assessed the effect of the AMPK-specific agonists 5-amino 4-imidazolecarboxamide riboside (AICAR) or metformin in newly plated hippocampal neurons (4). AMPK activation, as indicated by phosphorylation at Thr¹⁷², occurred rapidly and was reversible after incubation with AICAR or metformin. AMPK signaling could be blocked by a selective antagonist, compound C (CC), and was accompanied by phosphorylation of a major AMPK target, acetyl-coenzyme A-carboxylase (ACC) (Fig. 1, A to C). To examine AMPK activity on polarization, we treated cultured hippocampal neurons during the transition from stage 2 to stage 3, a critical period in which rapid axonal growth and polarization occur (5) (Fig. 1D). In cultures incubated with AICAR or metformin, we observed a significant reduction in the number of neurons possessing a typical elongated axon (Fig. 1, E to J). In addition, a mild or transient AMPK activation inhibited polarization (figs. S1 and S2). AICAR-treated neurons displayed no significant change in both average length and rate of growth of nonaxonal minor neurites (Fig. 1H and fig. S3, A to D), whereas the growth of axon-like neurites and total neurite length was markedly reduced (Fig. 1, I and J). Neurons treated immediately after plating also showed no defect in minor neurite formation or growth (fig. S3, E to H). The specific suppression of axonal, but not minor neurites, together with the lack of propidium iodide labeling (fig. S4), indicates that the AICAR effect did not result from cell damage.

To confirm that the effect on polarity was directly mediated via AMPK, we transfected neurons with either wild-type (WT) or a kinase dead (KD) mutant AMPK (D157A, mutation of Asp¹⁵⁷ to Ala) (Fig. 1, K and L). Overexpression of AMPK-WT alone caused a reduction in polarity that was further exaggerated by AICAR. In contrast, neurons expressing AMPK-KD established morphological polarity despite AICAR treatment, ruling out potential off-target effects of AICAR. Furthermore, expression of a constitutively active AMPK mutant (AMPK-CA) inhibited neuronal polarity and was not affected by AICAR (fig. S5). In line with the role of AMPK in axon initiation, progression into polarization stage 3 was accompanied by a drop in AMPK activity that remained stable throughout stage 3 (figs. S6 and S7). AICAR-induced AMPK phosphorylation was largely limited to the soma (fig. S8). Additionally, control cells displayed a mutually exclusive spatial distribution of MAP2 and Tau-1, markers for dendrite and axon, respectively. AICAR-treated neurons displayed a mixed distribution pattern (fig. S9), indicating that AMPK activity altered both molecular and morphological polarity. Because AICAR-induced AMPK activation is reversible (Fig. 1A), we assessed whether the effect of AMPK on polarity was tem-

porary. Neurons failed to establish polarity even 2 days after the removal of treatment (fig. S10), suggesting an irreversible effect. Furthermore, incubation of polarized stage 3 neurons with AICAR for 48 hours did not affect molecular polarity or axon length (fig. S11), excluding the possibility of a reversal in polarization.

The phosphatidylinositol 3-kinase (PI3K) signaling cascade is necessary for axonal neurite growth during polarization (6, 7). We wondered whether AMPK signaling might lead to an inhibition of the PI3K/Akt pathway. Instead, AICAR caused a substantial increase in total Akt phosphorylation (Fig. 2A and fig. S12), which was blocked by the PI3K inhibitor LY294002 (30 μ M) (Fig. 2B). LY294002 showed no effect on AICAR-induced AMPK activation, indicating that PI3K functions downstream of AMPK to regulate Akt activity.

During neuronal polarization, PI3K and Akt activity becomes selectively enriched at the tip of a single nascent neurite, signaling the progression from nonpolarized stage 2 to polarizing stage 3 (7). We reasoned that AMPK signaling might disrupt the subcellular localization of PI3K. In early stage 3 neurons, AICAR treatment induced a marked increase in pAkt immunointensity within the soma (Fig. 2, D and H). However, unlike control cells that showed enhanced pAkt dis-

tribution at the tip of a single neurite, AICAR-treated neurons revealed no tip enrichment (Fig. 2, D to H, and fig. S13). Similarly, single-tip enrichment of the PI3K regulatory subunit p85 (fig. S14) and the PI3K-regulated polarity protein mPar3 (7, 8) (fig. S15) was also abolished by AICAR. In addition, we examined the localization of the Akt-derived pleckstrin homology (PH) domain fused to enhanced green fluorescent protein (PH-EGFP) to indicate PI3K activity (9). Live imaging showed stable PH-EGFP localization at the tip of control axons, whereas 30-min AICAR treatment resulted in a retraction of PH-EGFP from the axon tip (Fig. 2J). Because PI3K acted downstream of AMPK (Fig. 2B), we attempted to rescue polarity by increasing PI3K activity. Most of the constitutively active PI3K (PI3K-CA) neurons polarized successfully despite enhanced AMPK activation (fig. S16), confirming the role of PI3K in AMPK-mediated polarity inhibition.

To explore whether AMPK activity alters motor-mediated tip transport of PI3K, we examined the involvement of kinesin I (Kif5), a plus end-directed molecular motor with an important role in axonal transport (10, 11) and cell polarity (12). Kif5 is also preferentially localized to the neurite tip of the future axon (13). Co-

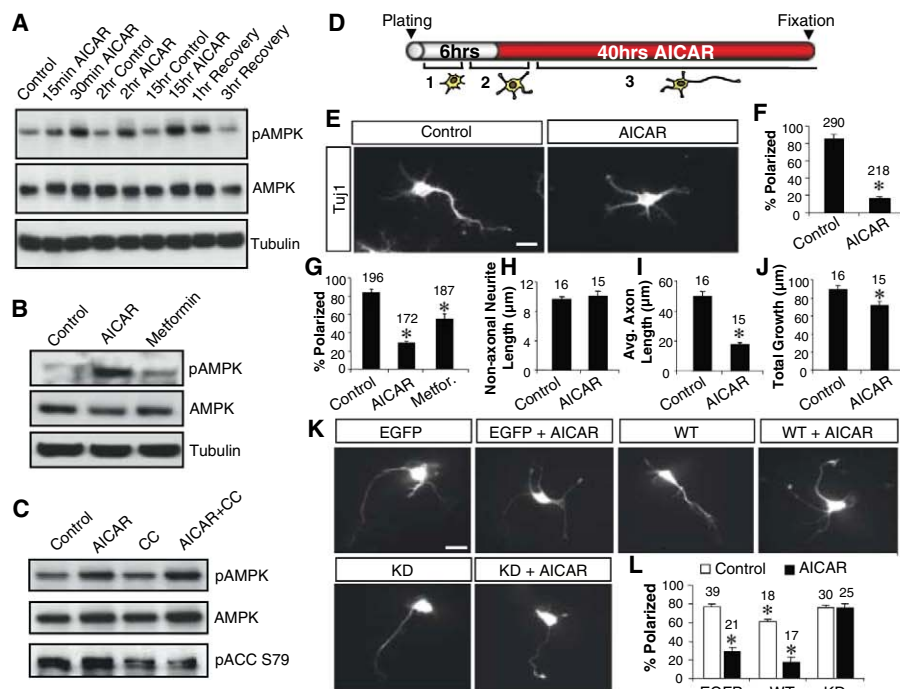


Fig. 1. AMPK activation suppresses neuronal polarization in cultured hippocampal neurons. **(A)** Time course for AMPK activation (pAMPK) by AICAR (2 mM) in day 1 in vitro (DIV1) hippocampal neurons. **(B)** Incubation of DIV1 neurons with AICAR and metformin (200 μ M) on AMPK activation. **(C)** Neurons were pretreated with compound C (CC) (20 μ M) for 1 hour followed by 30-min incubation with AICAR. **(D)** Illustration of AICAR treatment paradigm with corresponding polarization stages. **(E to G)** Effect of AMPK activation by AICAR and metformin on neuronal polarization. **(H to J)** Measurement of nonaxonal neurite length, axon length, and the total growth. **(K and L)** Expression of wild-type (WT) or kinase-dead (KD) AMPK on neuronal polarity. Cell numbers of statistical analysis are indicated at the top of bar graphs. * $P < 0.05$, two-population Student's t test [(F) and (H) to (J)], or analysis of variance (ANOVA) with Tukey post-test [(G) and (L)]. Scale bar, 20 μ m.

immunoprecipitation with stage 3 neuron lysates revealed an association of p85 with the cargo-binding kinesin light chain (KLC) (14–16), which was disrupted by AICAR (figs. S17A and S18). We identified two AMPK-dependent phospho-

rylation sites in KLC2, Ser⁵³⁹ and Ser⁵⁷⁵ (Fig. 3A and fig. S17, B and C). However, KLC2 double phosphorylation site mutation (KLC2-DM) failed to interact with p85 regardless of AMPK activation (Fig. 3B), perhaps due to tertiary-structure changes

that interfered with p85 binding (17). Nevertheless, expression of KLC2-DM resulted in a loss of p85 at the tip independent of AMPK activation, consistent with the inability of KLC2-DM to interact with PI3K (Fig. 3, C to E). Thus, KLC2

Fig. 2. AMPK activation disrupts tip localization of active Akt. (A and B) AICAR treatment on PI3K-mediated Akt phosphorylation in DIV1 neurons. (C) Illustration of AICAR treatment paradigm. (D) Hippocampal neurons treated with AICAR and LY294002 (LY), respectively, or together for 15 hours, immunostained for phospho-Akt (Ser⁴⁷³) and neuronal marker Tuj1. Scale bar, 20 μ m. (E) Quantification of tip fluorescence intensity normalized to shaft intensity. (F) Normalization of tip pAkt intensity to tip area indicated by EGFP. (G) EGFP intensity at axonal tip. (H) Quantification of pAkt soma intensity. (I) Plot profile of p-Akt signals from the soma to neurite tips. Dashed line indicates background intensity. (J) Live imaging of PH-EGFP in stage 3 neurons. * P < 0.05, ANOVA with Tukey post-test [(E) and (H)], or two-population Student's t test [(F) and (G)]. Scale bar, 10 μ m.

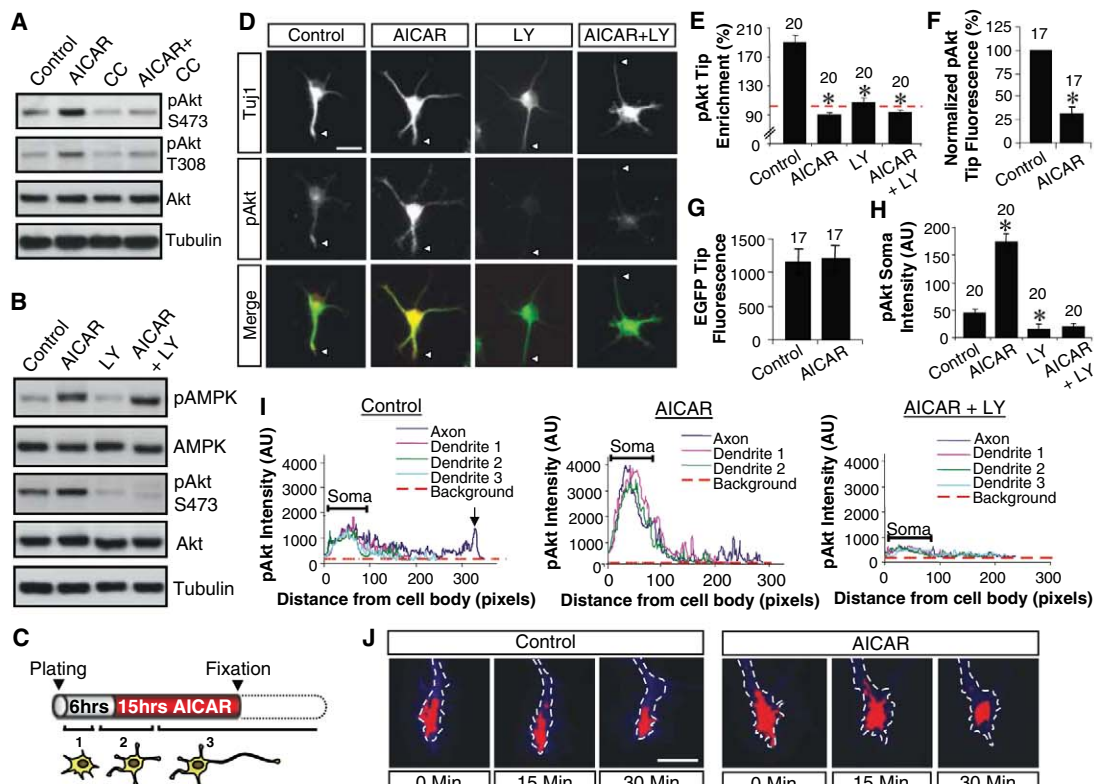
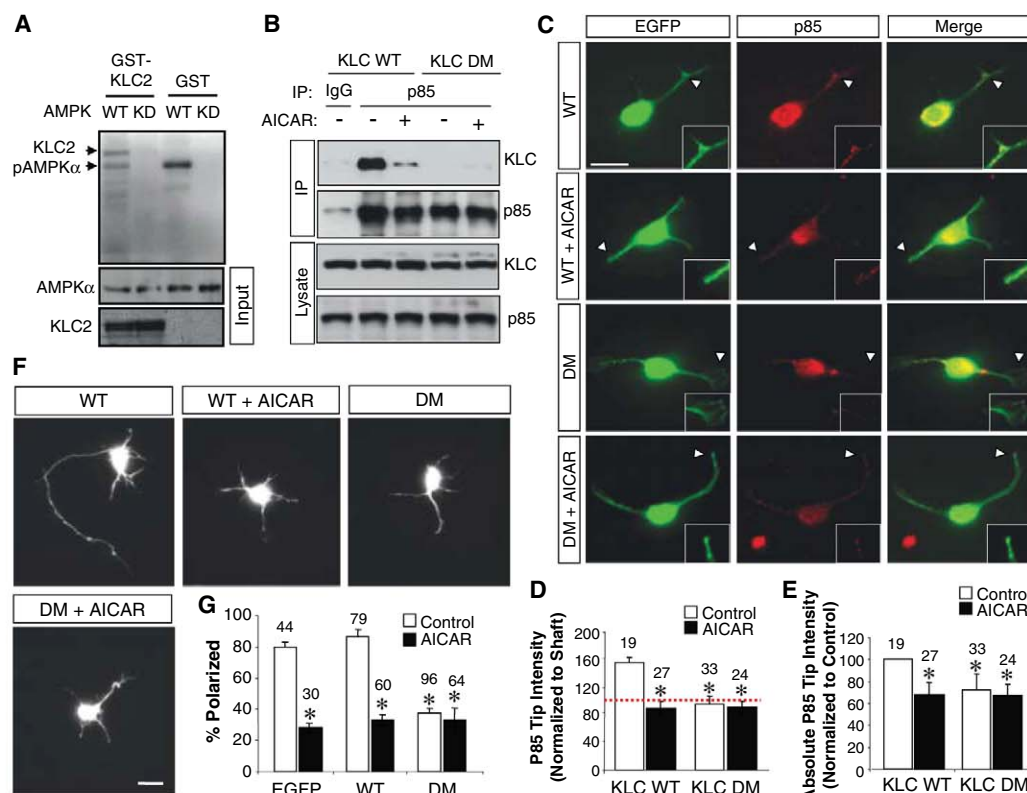


Fig. 3. Association of KLC2 and p85 is required for PI3K tip enrichment and neuronal polarity. (A) In vitro phosphorylation of KLC2 by AMPK. Purified GST-KLC2 was incubated with wild-type (WT) or kinase dead (KD) AMPK catalytic α 1 subunit in the presence of γ -³²P-ATP. Positive radioactivity was observed in KLC2 and AMPK α 1 subunit, indicating phosphorylation of KLC2 and autophosphorylation of AMPK. GST (glutathione S-transferase) was used as a control. (B) Immunoprecipitation of GFP-p85 in human embryonic kidney cells expressing GFP-p85 together with wild-type or mutant Myc-KLC2 (DM). (C to E) Immunostaining of p85 in stage 3 hippocampal neurons transfected with EGFP alone or together with either the WT or DM KLC2. (F and G) AICAR treatment on neurons expressing EGFP together with WT or DM KLC2. * P < 0.05, ANOVA with Tukey post-test. Scale bar, 20 μ m.



acts as a kinesin motor adaptor for PI3K transport to the axon tip. Consequently, neurons expressing KLC2-DM failed to establish polarity regardless of AMPK activation state (Fig. 3, F and G). Moreover, coexpression of PI3K-CA with KLC-DM rescued neuronal polarization to control levels (fig. S19), confirming that the KLC-DM effect was indeed due to a reduced PI3K activity at the neurite tip.

To determine the effect of AMPK activation on polarity in a more physiological setting, we prepared organotypic brain slice cultures from embryonic day 14 (E14) mice and used retroviral infection to express EGFP in newly divided neurons (18) (Fig. 4A). In brain slices, AICAR incubation induced similar activation of AMPK and Akt pathways (fig. S20). In control slices,

GFP-expressing neurons showed a typical basal axon and multiple apical dendrites derived from a single dendritic base (Fig. 4B). AICAR-treated neurons displayed no axon or possessed only a very short rudimentary neurite at the axonal pole and atypical dendrite patterning (Fig. 4, C to F). To further confirm the role of AMPK activity, we electroporated E15.5 mouse cortex in utero with $\text{T}\alpha\text{-1}$ Venus EGFP combined with AMPK-KD (Fig. 4, G to M). There was no discernable difference between neurons expressing EGFP alone and those coexpressing AMPK-KD. AICAR-treated neurons were characterized by several dendritic processes grown directly from the soma protruding in multiple directions and by irregular cell bodies, hallmarks of multipolar nonpolarized

neurons in vivo (19–21). In contrast, a polarized morphology in AMPK-KD-expressing neurons was successfully established, with typical axon-dendrite bipolar neurites and a more elongated soma, despite AICAR treatment. Additionally, an ischemic challenge in cultured neurons induced an AMPK-dependent suppression of cell polarization (fig. S21, A to D), supporting an involvement of this energy-sensing pathway in pathophysiological conditions.

Here we have identified a crucial role of the bioenergy-sensing pathway in axon initiation, neuronal polarization, and differentiation into axons or dendrites. The AICAR effect on polarization was abolished by introduction of kinase-dead AMPK, suggesting the requirement for AMPK activation. In native tissues and the brain, liver kinase B1 (LKB1) and calmodulin-dependent protein kinase kinase β (CaMKK β) are major upstream activators of AMPK (22). LKB1 mediates axon initiation through SAD kinase activation (23, 24). However, LKB1 knockout had no effect on AMPK phosphorylation within the cortex (23). Furthermore, suppression of CaMKK activity inhibits neurite growth (25, 26). Thus, the upstream signaling molecules involved in the observed AMPK effect remain unclear. Because AMPK activity is coupled to AMP/ATP ratio, regulation of AMPK by the upstream kinases should vary depending on energy status. In addition, given the large number of downstream targets, LKB1- or CaMKK β -dependent cellular responses can be attained with substrates other than AMPK signaling. Indeed, transforming growth factor- β (TGF- β) signaling can lead to a similar phenotype in neuromorphogenesis (27), indicating the existence of multiple signaling pathways for neuronal polarization.

The AMPK effect on polarity was accompanied by a loss of axon-tip enrichment of several signaling components, including PI3K, p-Akt, and mPar3. In contrast to a reduced function at the neurite tip, PI3K and Akt activities at the total cellular level were greatly increased during AMPK activation. Under reduced-energy conditions that lead to AMPK activation, entry of glucose should be facilitated in order to increase ATP production (28). Perhaps the observed increase in PI3K/Akt activity during AICAR treatment facilitates increased glucose uptake (29). PI3K dislocation from the neurite tip may result from a failure in its motor-mediated delivery, due to AMPK-dependent phosphorylation on KLC. PI3K might be transported via direct P85-KLC interaction, similar to the delivery of collapsin response mediator protein-2 (CRMP-2) (15), or via indirect association through an intermediate molecule.

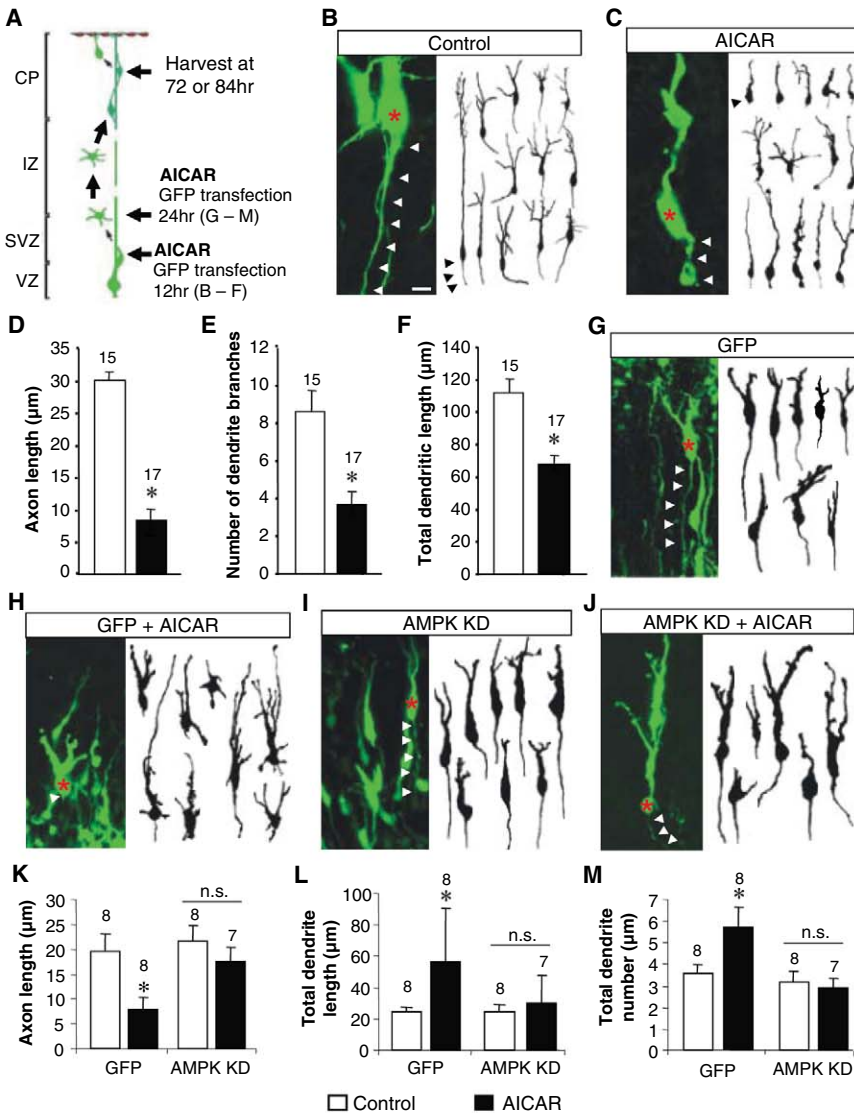


Fig. 4. AMPK activation disrupts neuronal polarization in organotypic slice culture. (A) Illustration of experimental design and stages of neuronal polarization. (B to F) Cortical brain slices prepared from E14 mice were infected with CAG-GFP retrovirus to indicate newborn neurons and treated with AICAR (2 mM, 72 hours). (G to M) The embryonic brain (E15.5) was in utero-electroporated with EGFP with or without AMPK-KD, and brain slices were prepared for AICAR incubation (48 hours). Arrowheads and stars indicate axonal segment and the soma, respectively. * $P < 0.05$, two-population Student's t test [(D) to (F)], or ANOVA with Tukey post-test [(K) to (M)]. Scale bar, 10 μm .

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Materials and Methods

Figs. S1 to S21

References and Notes

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Coping with Chaos: How Disordered Contexts Promote Stereotyping and Discrimination

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Being the victim of discrimination can have serious negative health- and quality-of-life-related consequences. Yet, could being discriminated against depend on such seemingly trivial matters as garbage on the streets? In this study, we show, in two field experiments, that disordered contexts (such as litter or a broken-up sidewalk and an abandoned bicycle) indeed promote stereotyping and discrimination in real-world situations and, in three lab experiments, that it is a heightened need for structure that mediates these effects (number of subjects: between 40 and 70 per experiment). These findings considerably advance our knowledge of the impact of the physical environment on stereotyping and discrimination and have clear policy implications: Diagnose environmental disorder early and intervene immediately.

There is substantial evidence that discrimination has serious negative consequences for those who are discriminated against, as well as for society in general (1–3). A neglected possible source of stereotyping and discrimination is physical disorder. The environment can affect the relative accessibility of important goals (4, 5), and recently it has been found that physical disorder in particular can, through shifting the relative accessibility of goals, increase littering, trespassing, and even stealing (6). But can physical disorder also lead to increased stereotyping and even discrimination? We have reason to believe that it does, because physical disorder is likely to increase the need for structure, thereby boosting the goal to create order, leading to the use of highly simplified categories and judgments (stereotypes), which, in turn, may trigger discriminatory behavior.

There is some evidence that stereotyping is goal-driven (7–9), and there is even evidence that

when people's desire for structure and predictability is high, they are more likely to engage in stereotyping than when it is low (10–13). Thus, disorder can be expected to increase the need for structure and make the goal to perceive order more salient, a goal that can, at least temporarily, be satisfied by stereotyping. Seen in this light, stereotyping is a way to cope with chaos, a mental cleaning device in the face of disorder. In many situations, discrimination is a likely correlate of stereotypes. Stereotypical behaviors are often activated along with stereotypical traits, (14) and they can be discriminatory in the dealings with members of lower-valued outgroups. Members of such outgroups are linked to many negative stereotypical traits that are salient in public encounters (such as danger or contagiousness), in evaluative situations (such as laziness or incompetence), and in situations of neediness (such as unworthiness or undeservedness) (15). Linked to these stereotypical traits are stereotypical reactions, such as keeping one's distance, excluding someone from membership or position, or declining help to someone in need (16).

We tested our hypothesis about the impact of disorder on stereotypes and their discriminatory behavioral correlates in a series of field and lab experiments, using a variety of explicit and covert disorder primes and stereotyping measures. In

our two field experiments, we tested the impact of real-world situations of disorder on stereotyping and its behavioral correlates. In the three lab experiments, we subsequently tested the proposed mechanism itself. In all experiments, we tested for effects of participants' gender and mood. Because we did not find any significant effects of these two variables, we will not report them in the remainder of this article (17).

In our first field experiment, we interviewed travelers in a train station. In this experiment the dependent variable consisted of a judgmental measure (a survey of trait judgments about some social groups) and a behavioral measure (discrimination measured as physical distance from a member of an ingroup versus outgroup while filling out the survey). We predicted that in a dirty train station people stereotype more and would choose to sit further away from an outgroup confederate than in a (relatively) clean train station. A recent strike by the cleaners of Utrecht train station in the Netherlands provided a unique opportunity to test the impact of considerable physical disorder on stereotyping against the impact of physical orderliness in the same public location. Utrecht station is a train hub in the middle of the Netherlands, where thousands of travelers pass through on a daily basis. Thus, during the cleaners' strike, the train station quickly turned into a dirty and disordered environment. After the station had not been cleaned for a few days in a row, we asked 40 travelers [mean age = 32 years; 50% female, all Caucasian (17)] who were waiting for their train to participate in this study in return for a candy bar or an apple. They were asked to judge (on a nine-point scale, ranging from 1 = not at all to 9 = very much) the extent to which they thought certain traits applied to a particular group (in our case, Muslims, homosexuals, and the Dutch) (17).

To get a behavioral measure of discrimination related to stereotyping, travelers were asked to fill out the short questionnaire in an area where there were six chairs lined up. Respondents (who were all Caucasians) could choose any chair, except that the first chair in the row was already taken by either a black (Dutch-African) or white (Dutch-Caucasian) confederate. Through random assignment, for half of the participants this confederate

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was a 20-year-old male Dutch-African (black) person, and for the other half, the confederate was a (20-year-old) male Dutch-Caucasian (white) person. Pretests had shown that these two confederates were judged as equally intelligent, friendly, attractive, and approachable. The dependent variable was the distance in number of chairs (zero to four) between the chair with the confederate and the chair the participant chose to sit on. A week later, after the cleaners had returned to work and the train station looked clean and orderly again, we returned and repeated the experiment (again with 40 subjects, 50% female, all Caucasian).

For the purpose of presentation and because, as expected, the pattern of means for the positive- and negative-stereotyping measures (18) was very similar for each of the three social groups, we combined the means in one stereotyping judgment score ($\alpha = 0.76$). An analysis of variance showed the predicted effect of environment on stereotyping: $F_{1,78} = 13.11$, $P < 0.01$. When the train station had not been cleaned for several days, participants stereotyped significantly more (mean $M = 5.12$, standard deviation $SD = 1.01$) than when it looked nice and clean ($M = 4.28$, $SD = 1.03$). Thus, people's tendency to use stereotypes in their social judgments clearly increased with physical disorder.

Importantly, this stronger stereotyping in the disorder condition was accompanied by a significant increase in the distance the respondents chose to put between themselves and the black confederate, compared with the order condition (see Fig. 1): $F_{1,78} = 7.23$, $P < 0.01$. When the train station had been cleaned, however, participants' choice of seat was not significantly affected by the confederate's ethnicity ($F < 1$). This clearly suggests that the tendency for behavior to be driven by preexisting knowledge structures (18), such as representations of stereotypical reactions ("distance yourself from people belonging to group X"), increases with physical disorder. In a disordered environment, people are more likely to distance themselves from outgroup members than in a clean and ordered environment.

Were the results of this first study actually due to disorderliness, or were they rather a reaction to "uncleanness"? In the second field experiment, we tried to address this issue by manipulating environmental disorder while keeping cleanliness constant (17). We did this by approaching 47 passersby (mean age = 42; 57% female, all Caucasian) on a street in an affluent neighborhood in a Dutch city and asking them to fill out a stereotyping questionnaire (see first field experiment) in return for 5 Euros. Disorder was manipulated by some subtle environmental interventions. We took out and misplaced some of the tiles in the pavement of the sidewalk, put a badly parked car (with two wheels on the sidewalk, windows open) near the spot where respondents were interviewed, and put a bicycle on the street, as if it had been abandoned. When respondents had completed the questionnaire, they were given 5 euros (three

coins of 1 euro, four coins of 50 eurocents), and asked to donate (some of) that money to the "Money for Minorities" fund (identified as an initiative to help members of minority groups—such as immigrants, homeless people, and people from other countries—to live better and successful lives). The amount of contribution to this fund (0 to 5 euros) was our behavioral measure. In the control condition (that was run a day later), the same pavement, car, and bicycle were there, but everything looked nice and neat. The prediction was that, compared to a control condition, respondents stereotype more and donate less money for minorities when they are interviewed in a disorderly environment. As expected, an analysis of variance revealed that disorder respondents ($n = 24$) scored significantly higher on the stereotyping measure ($M = 5.07$, $SD = 0.98$) than did control participants ($n = 23$; $M = 4.29$, $SD = 1.00$): $F_{1,45} = 7.17$, $P < 0.01$. The analysis also showed, as predicted, that people who were in a disordered environment gave significantly less money to the Money for Minorities fund ($M = 1.70$, $SD = 0.96$) than did participants who were in the control condition ($M = 2.35$, $SD = 0.93$): $F_{1,45} = 5.71$, $P < 0.05$. Thus, the results of the second field experiment support the results of the first field experiment.

The results of the two field experiments show the same pattern, but are they really due to differences in the need for structure and, thus, differences in the relative weight of the goal to create order? In the next experiment, we addressed this question directly. We first exposed people to a series of four pictures of events and scenes depicting disorder (e.g., a book case with chaotically stacked books), order (e.g., a book case with nicely stacked books), or neutral pictures (e.g., pictures of a table, chair, or ball). We predicted that disorder would lead to more stereotyping and that an increase in the need for structure is an important force behind this effect (17). Forty-seven students (mean age = 20, 64% females, all Caucasian) were randomly assigned to a disorder,

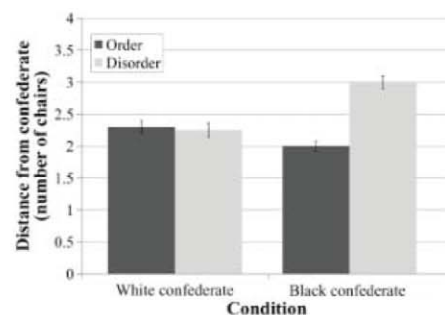


Fig. 1. Behavioral measure of discrimination (with error bars indicating SE). The distance subjects chose to put between themselves and the Dutch-Caucasian (white) or Dutch-African (black) confederate (measured in number of chairs) as a function of an orderly (clean) or disorderly (trash-littered) environment.

order, or neutral priming condition. Participants were asked to look at the set of four pictures and become familiar with them and to be prepared to answer questions about the pictures later in the session. After participants had finished this task, in an ostensibly different study, their need for structure was assessed using eight items of the personal-need-for-structure scale (18, 19) ["It annoys me when I get into situations in which I do not know what to expect," "I do not like situations that are uncertain," "I need structure," modeled after (17) and (18)] on nine-point scale (1 = completely disagree, 9 = completely agree) ($\alpha = 0.77$). Finally, stereotyping judgments were measured as before (see field experiments). As can be seen in Fig. 2, both the need for structure and stereotyping are considerably higher in the disorder condition than in the order or neutral conditions. These effects are significant [$F_{2,44} = 7.06$, $P < 0.01$ and $F_{2,44} = 4.48$, $P < 0.05$, respectively].

In support of the hypothesis that the need for structure mediates the impact of disorder on stereotyping, we found that the need for structure and the need for stereotyping were highly correlated ($r = 0.69$, $P < 0.01$). Next, a covariance analysis indicated that the effect of condition on stereotyping was no longer significant when the need for structure was included as a covariate: $F_{2,43} = 1.10$, $P = 0.34$ (t test covariate = 7.57, $P < 0.01$). In addition to this covariance analysis, we used the bootstrapping method for testing mediation proposed by Preacher and Hayes (20) to test to what extent structure striving and/or cognitive load mediated the effects of disorder on stereotyping. This analysis revealed the need for structure (and not cognitive load) as a significant mediator for the effect of disorder on stereotyping [see supporting online material (SOM) for detail] (17).

The first lab experiment shows the stereotyping effect for scenes of disorder. Would the effect on stereotyping also hold for unconscious exposure to concepts of disorder? We conducted a second lab experiment to find out (17). Fifty-eight students (mean age = 20, 67% females, all Caucasian) were randomly assigned to a disorder,

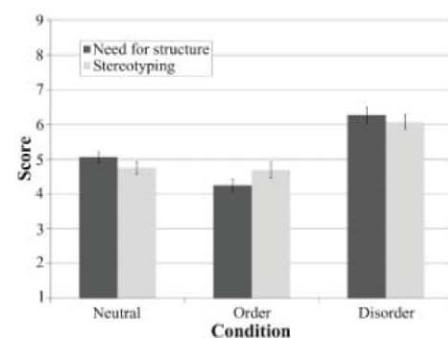


Fig. 2. Need for structure and degree of stereotyping (with error bars indicating SE) as a function of order condition (e.g., a neutral picture with a table, a picture of an orderly stacked book case, a picture of a chaotically stacked book case).

order, or neutral priming condition in a parafoveal vigilance task in which words were presented outside of awareness (21). The words that were presented in 20 of the disorder and order trials and in all of the neutral priming trials, were neutral words (e.g., table, chair, ball). In the remaining 40 disorder trials, disorder words (e.g., chaos, anarchy, mess) or order words (e.g., structure, clarity, neat) were presented. We used the same measures for need for structure and stereotyping as in the first lab experiment. The results of the analysis of variance looked almost identical to those of the first lab experiment. Disorder participants reported a significantly higher need for structure ($M = 6.16$, $SD = 1.54$) than both order ($M = 4.29$, $SD = 1.88$) and neutral participants ($M = 4.67$, $SD = 1.09$): $F_{2,55} = 7.90$, $P < 0.01$. Disorder respondents also scored significantly higher on the stereotyping measure ($M = 5.95$, $SD = 1.13$) than both order ($M = 4.38$, $SD = 1.50$) and neutral participants ($M = 4.89$, $SD = 1.32$): $F_{2,55} = 7.09$, $P < 0.01$.

In support of the hypothesis that the need for structure mediates the impact of disorder on stereotyping, we found that the need for structure and stereotyping were highly correlated ($r = 0.77$, $P < 0.01$). Next, a covariance analysis indicated that the effect of condition on stereotyping was no longer significant when the need for structure was included as a covariate: $F_{2,55} = 1.13$, $P = 0.33$ (t test covariate = 6.02, $P < 0.01$).

A crucial element in the mechanism we suggested is that the act of stereotyping does reduce the need for structure (22, 23). Is this indeed the case? If so, the effect should also hold for disorder per se and not depend on meaningfulness (such as trash, broken street tiles, the word "anarchy," etc). In our third and final lab experiment, we set out to address this issue as follows: After order versus disorder was primed, participants completed a filler task or a stereotyping measure, and their need for structure was assessed. The prediction was that even abstract disorder increases the need for structure and that stereotyping can satisfy this need. Thus, the need for structure should be lower when a disorder prime is followed immediately by a stereotyping measure, compared to when people have not been given the opportunity to stereotype.

Upon arrival in the room where the study took place, participants (all students, $n = 66$, mean age = 20, 50% female, all Caucasian) were given a sheet of paper on which a number of circles, squares, and triangles were displayed. In the disorder condition, these symbols were displayed randomly and chaotically across the page; in the order condition, these symbols were displayed in a neat and symmetric pattern. Pretesting had revealed that the disordered figure was indeed perceived as more "chaotic" and "random" than the ordered figure. Participants were asked to look at the pictures, become familiar with them, and be prepared to answer questions about the pictures later in the session. Next, participants were given either the stereotyping measure or a

filler task (rating the attractiveness of a number of Western European cities), and their need for structure was subsequently assessed. Results of the analysis of variance show that even disorderly abstract patterns can increase stereotyping. We observed significantly higher stereotyping in the disorder condition ($M = 5.62$, $SD = 0.83$) compared with the order condition ($M = 4.40$, $SD = 0.99$): $F_{1,28} = 13.03$, $P < 0.01$. We also found, as expected, that stereotyping satisfies the need for structure. In the disorder condition, need-for-structure scores were significantly higher when participants had not been able to engage in stereotyping ($M = 6.14$, $SD = 1.35$) than when they did have this opportunity ($M = 5.13$, $SD = 0.99$). When order was primed, the need for structure was relatively low, independent of whether or not people could engage in stereotyping ($M = 5.33$, $SD = 1.05$; versus $M = 4.88$, $SD = 1.09$). This interaction was significant: $F_{1,56} = 6.40$, $P < 0.05$.

People are very sensitive to their experience of disorder. In five studies, we obtained evidence that there are important links between the experience of disorder, the need for structure, and stereotyping/discrimination. Environments that are perceived as disordered invite people to apply stereotypes in their reactions to others more readily than do environments that are perceived as relatively orderly and structured. This was demonstrated in field as well as in lab experiments, using a variety of methods to activate perceptions of disorder (or order) and using judgmental as well as behavioral measures of the stereotyping tendencies. We interviewed people in a dirty (or clean) train station and in a disorderly (or orderly) looking street, and we primed people with pictures, words, and abstract symbols that represent disorder or order. We consistently found that disorder activated the tendency to stereotype and to discriminate others. Moreover, we also provided strong evidence for the mechanism behind this effect: Environmental cues can temporarily change the relative weight of an important goal (4, 6); in this case, the goal to create order. The findings reported here clearly show that the disorder-to-stereotyping effects were not driven by a lack of cleanliness itself, but by disorder affecting the need of structure. In the SOM that supports this article, we report an experiment with strong evidence that these effects can also not be explained in terms of disorder increasing the use of stereotypes by increasing cognitive load. In this additional experiment, we show that cognitive load also increases the need for structure and that load-based stereotyping effects (24) may best be explained in terms of changes in structure striving (17).

Our studies show that disorder increases the need for structure and, thus, the goal to create order. The study also shows that stereotyping is an effective mental way to reach this goal; that is, to satisfy the desire for structure that is activated by physical disorder. Stereotyping is a mental cleaning device that helps people to cope with physical chaos.

Effects of disorder on stereotyping and discrimination can occur virtually everywhere: in neighborhoods, public places, public transportation, hospitals, schools, firms, sport clubs, and arenas. Thus, the message for policy-makers is clear: One way to fight unwanted stereotyping and discrimination is to diagnose environmental disorder early and to intervene immediately by cleaning up and creating physical order. Signs of disorder such as broken windows, graffiti, and scattered litter will not only increase antisocial behavior (6), they will also automatically lead to stereotyping and discrimination. Thus, constantly avoiding that such signs become salient, investing in repair and renovation, and preventing that neighborhoods fall into disarray, may be relatively inexpensive and effective ways reduce stereotyping and discrimination.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6026/251/DC1
SOM Text
References

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Rapid Spread of a Bacterial Symbiont in an Invasive Whitefly Is Driven by Fitness Benefits and Female Bias

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Maternally inherited bacterial symbionts of arthropods are common, yet symbiont invasions of host populations have rarely been observed. Here, we show that *Rickettsia* sp. nr. *bellii* swept into a population of an invasive agricultural pest, the sweet potato whitefly, *Bemisia tabaci*, in just 6 years. Compared with uninfected whiteflies, *Rickettsia*-infected whiteflies produced more offspring, had higher survival to adulthood, developed faster, and produced a higher proportion of daughters. The symbiont thus functions as both mutualist and reproductive manipulator. The observed increased performance and sex-ratio bias of infected whiteflies are sufficient to explain the spread of *Rickettsia* across the southwestern United States. Symbiont invasions such as this represent a sudden evolutionary shift for the host, with potentially large impacts on its ecology and invasiveness.

The life history traits of multicellular organisms, generally thought of as fundamental properties of the organism themselves, are often influenced by interactions with symbiotic species (1–4). These symbiont associations may form enduring evolutionary relationships with their hosts but can also be dynamic, entering and leaving populations in spans of a few years to thousands of years (5, 6). The maternally inherited, endosymbiotic bacteria of insects comprise some of the most intimate of these associates. These symbionts spread in host populations either by increasing the fitness of all infected hosts or by manipulating host reproduction in ways that increase maternal transmission of the sym-

biont. Although the former is clearly beneficial to the host, the female-biased sex ratios or reproductive incompatibility caused by the latter are not in the host's genetic interests and are considered reproductive parasitism (7). Generally symbionts fall clearly into one of these categories and/or symbiont spread can be clearly assigned to one or the other function [e.g., (8)]. Here we describe the rapid spread of a bacterial symbiont, *Rickettsia* sp. nr. *bellii*, in a population of an invasive whitefly, *Bemisia tabaci*. *Rickettsia* infection increases whitefly performance; infected whiteflies produce more offspring that survive to adulthood at greater rates and develop more quickly. Symbiont infection also causes a strong female bias. Our results provide evidence of a rapid symbiont invasion in nature driven by the simultaneous expression of two host phenotypes, both contributing to symbiont spread.

Intracellular bacterial symbionts are common in insects. For example, *Wolbachia* is estimated to infect 66% of all insects (9), and ~10% of insect species have obligate nutritional symbionts that provide essential nutrients to their insect hosts (10). The endosymbiotic α -proteobacteria in the genus *Rickettsia* are best known for causing arthropod-vector human diseases such as typhus

and Rocky Mountain spotted fever (11). However, recent surveys have uncovered a diversity of *Rickettsia* in arthropod hosts that are not blood feeders (12, 13). In the majority of these cases, the role of *Rickettsia* is unknown [e.g., (14)], but they may cause plant disease or manipulate arthropod host reproduction in ways that enhance transmission. For example, different *Rickettsia* species cause male-killing in a coccinellid beetle and parthenogenesis in parasitoid wasps (12, 15).

The host of *Rickettsia* sp. nr. *bellii* in the current study is the sweet potato whitefly, *B. tabaci* (Hemiptera: Aleyrodidae). This sap-feeding insect is cosmopolitan and one of the worst pests of tropical and warm-temperate agriculture worldwide (16). *B. tabaci* is a species complex composed of several populations or biotypes that are genetically distinguishable, are more or less reproductively isolated, and differ in a wide range of biological characteristics, including invasiveness (17). These “biotypes” house distinct symbiont complements (18, 19). In addition to *Portiera aleyrodidarum*, the obligate nutritional symbiont that is thought to supplement the amino acid-poor diet (20), populations of *B. tabaci* contain different suites of facultative symbionts, including species of *Wolbachia*, *Cardinium*, *Hamiltonella*, *Fritschea*, *Arsenophonus*, and *Rickettsia* (19). The B biotype, the dominant *B. tabaci* biotype in the United States and the subject of this study, is fixed for both *P. aleyrodidarum* and *Hamiltonella defensa* (18, 21). The roles of these facultative symbionts are largely unknown but likely contribute to the biological differences observed among whitefly biotypes (19).

Here, we report the rapid spread of *Rickettsia* sp. nr. *bellii* (referred to as *Rickettsia* hereafter) in *B. tabaci* in the southwestern United States. Preserved samples of whiteflies from several sites in Arizona, New Mexico, and California show that the frequency of infection rose from 1% in 2000 to 51% in 2003 and to 97% in 2006 (Fig. 1) (22). Samples taken in 2008 and 2009 indicate that infection rates continue at near fixation. Given that *B. tabaci* may complete as many as 13 generations per year in the desert southwest (23), the spread from low *Rickettsia* frequency to near fixation occurred in less than 80 generations. This speed of increase is comparable to, or faster than, known examples such as the *Wolbachia* invasion

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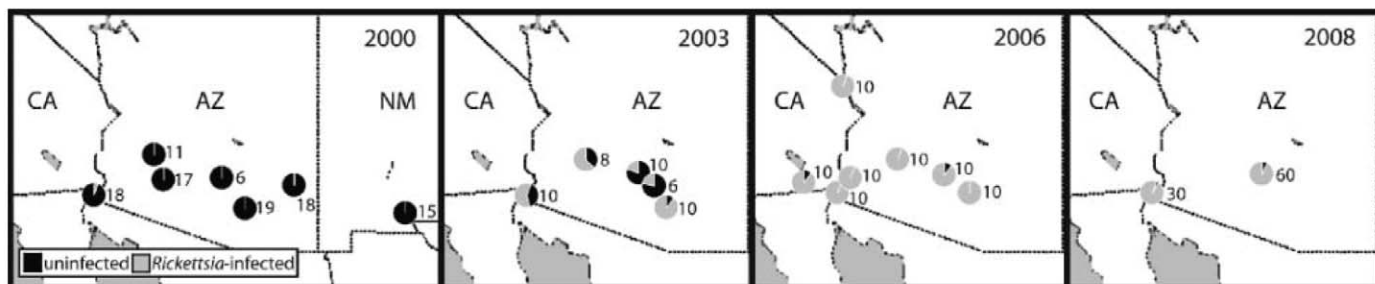


Fig. 1. Spread of *Rickettsia* in *B. tabaci*. The black proportion of circles represents uninfected whiteflies, and gray represents *Rickettsia*-infected whiteflies. Numbers indicate sample size. Infection rate spread from 0.01 in 2000 ($N = 103$), to 0.51 in 2003 ($N = 47$), to 0.97 in 2006 ($N = 70$), and to 0.94 in 2008 ($N = 90$).

of *Drosophila simulans* documented in California by Turelli and Hoffmann (6).

To determine whether *Rickettsia* also spreads in the laboratory, the frequencies of *Rickettsia* were followed over five whitefly generations on three host plants (cotton, melon, and cowpea). The infection spread quickly when initiated at 14% of hosts infected (Fig. 2) (Wald's $\chi^2_{\text{time}, 2 \text{ df}} = 59.48$, $P < 0.0001$; Wald's $\chi^2_{\text{time} \times \text{host plant}, 4 \text{ df}} = 31.22$, $P < 0.0001$). These data show that spread can occur on whitefly populations reared on at least three different host plants in the laboratory and is not limited by factors that occur only in the field, such as a particular host plant or natural enemy.

How did *Rickettsia* spread? After an introgression series of crosses to homogenize genetic background (22), we examined the rates of both vertical and horizontal transmission of *Rickettsia* in whiteflies. Experiments showed near perfect vertical transmission of *Rickettsia* from infected mothers to daughters (99.17% infected; $n = 120$ daughters from 18 mothers). In contrast, the rate of horizontal transmission from infected males to uninfected females on the same plant did not differ significantly from 0% (median = 0% infection for 8 replicates) (22). Therefore, subsequent experiments compared the performance of *Rickettsia*-infected (R^+) and uninfected (R^-) whiteflies.

Rickettsia-infected whiteflies on cowpea leaf disks produced almost double the number of adult progeny over their lifetimes (Fig. 3A) ($t_{47 \text{ df}} = 4.55$, $P < 0.0001$), and their offspring had greater survival to adulthood relative to uninfected whiteflies (Fig. 3B) (Wald's $\chi^2_{1 \text{ df}} = 14.37$, $P < 0.001$). Moreover, R^+ females produced a higher proportion of daughters than R^- whiteflies, which would also contribute to *Rickettsia* spread (Fig.

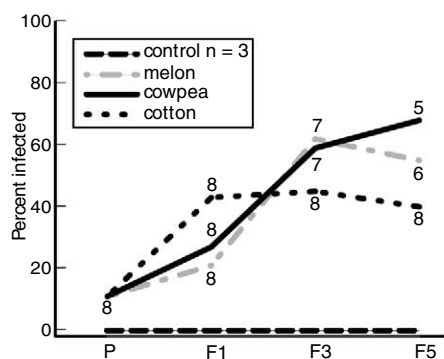


Fig. 2. Spread of *Rickettsia* infection in population cages of whiteflies over five generations on three host plants from 14% starting frequency. Numbers indicate the number of replicate cages per plant type at each generation. Control cages were inoculated only with R^- whiteflies and served as a check to detect possible contamination. A general linear model analysis showed that frequencies of infection changed significantly over time ($P < 0.0001$) and that the host plant influenced frequencies in interaction with time ($P < 0.0001$).

3C) (Wald's $\chi^2_{1 \text{ df}} = 9.52$, $P < 0.002$). An experiment with cohorts of whiteflies on whole cowpea plants produced similar results (Fig. 3, D to F). *Rickettsia*-infected females produced more juvenile progeny (Fig. 3D) ($F_{1,6} = 53.37$, $P < 0.0003$), had greater survival to adulthood (Fig. 3E) (Wald's $\chi^2_{1 \text{ df}} = 93.52$, $P < 0.0001$), and had more female-biased sex ratios (Fig. 3F) ($\chi^2_{1 \text{ df}} = 9.64$, $P < 0.002$) than uninfected females. In addition, the latter experiment showed R^+ females and males develop 1 to 2 days faster than R^- whiteflies (Fig. 4) ($t_{\text{females}, 14 \text{ df}} = 5.82$, $P < 0.0001$; $t_{\text{males}, 14 \text{ df}} = 2.37$, $P < 0.05$).

To determine whether the benefits of infection observed in the lab were sufficient to explain the rate of spread measured in the field, we used a simple growth model that incorporated the effects of infection on whitefly fecundity, survival, and sex ratio, as measured in two separate laboratory experiments (22). Using the data from either of the two experiments, the results from the model indicate that the observed effects of infection were sufficient to explain the rate of spread in the field (22). The rate of spread predicted from the model was higher than that seen in the field, suggesting that the spread in the field was slowed by one or more factors not included in the model (22).

How *Rickettsia* influences performance and sex ratio of whiteflies is unknown. Among possible mechanisms are manipulation of plant quality by the *Rickettsia*-infected whiteflies; plant quality

can dramatically change whitefly performance (24). Alternatively, *Rickettsia* could serve as a nutritional mutualist or a defensive mutualist against cryptic pathogens present in the field and laboratory [e.g., (8, 25)].

Our results contrast with previous work from Israel that showed slight performance benefits (R^+ whiteflies developed faster than R^-) but no reproductive manipulation associated with *Rickettsia* infection in the B biotype of *B. tabaci* (26). The reason for this disparity is unclear, but whiteflies in Israel also show lower *Rickettsia* infection frequencies in the field (18), suggesting a fundamental difference between the Israeli and U.S. whitefly-*Rickettsia* interactions.

The results of this study show both host reproductive manipulation (strong female bias) and large fitness benefits associated with a single symbiont lineage. Multiple roles have recently been discovered for a few symbionts. A *Wolbachia* strain previously shown to cause weak reproductive incompatibility in *Drosophila melanogaster* (27) was shown to confer strong virus resistance (8). More commonly, reproductive manipulator symbionts have been associated with modest increases in performance relative to uninfected individuals, likely as a result of coevolution of host and symbiont lineages [e.g., (28, 29)].

The sex-ratio bias and performance benefits associated with *Rickettsia* infection in the current study both provide an explanation for the sweep of *Rickettsia* in the field and in the laboratory.

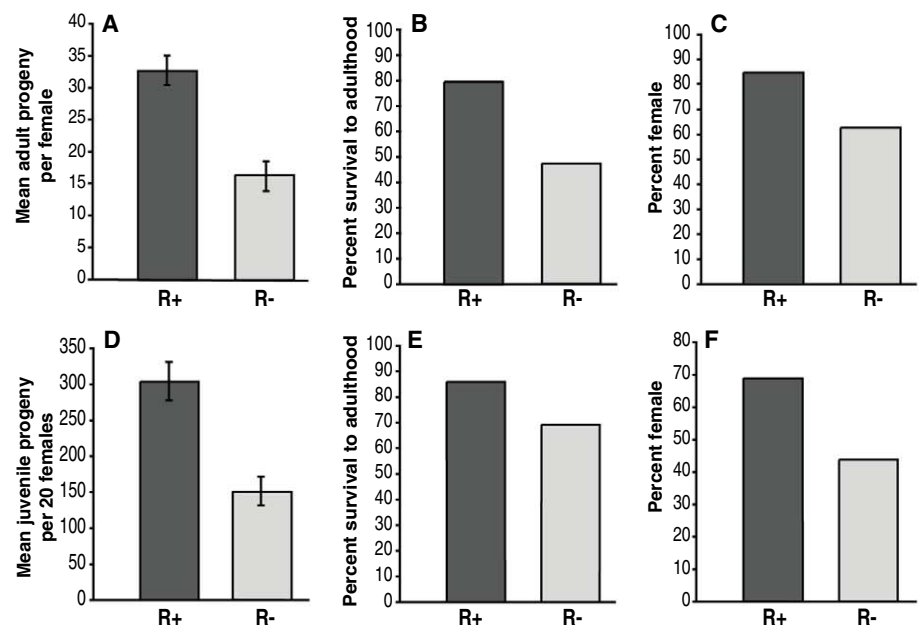


Fig. 3. (A to C) Life history parameters of R^+ and R^- whiteflies on cowpea leaf disks. (A) Mean adult progeny produced per female after a 3-day oviposition period $\pm$ SEM ($P < 0.0001$). (B) Percentage survival to adulthood ($P < 0.001$). (C) Sex ratio (percentage female, $P < 0.002$). (D to F) Life history parameters of R^+ and R^- whiteflies on whole cowpea plants. (D) Mean juvenile progeny produced per cohort of 20 females after a 5-day oviposition period $\pm$ SEM ($P < 0.0003$). (E) Percentage survival to adulthood ($P < 0.0001$). (F) Sex ratio (percentage female, $P < 0.002$). Sample sizes were 21 to 29 individual females per treatment for (A) to (C) and eight replicates per treatment of 20 females each for (D) to (F) (22).

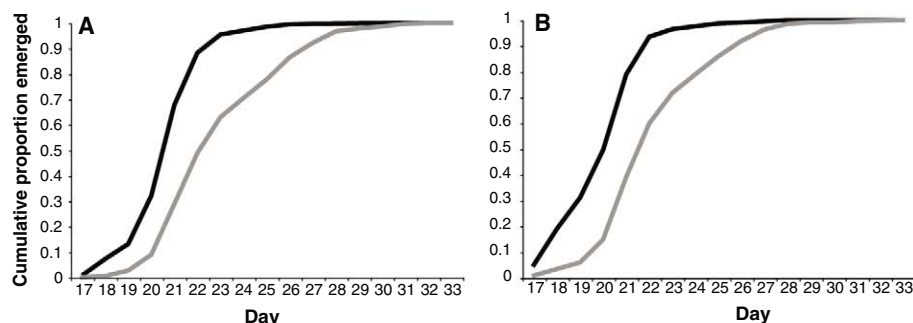


Fig. 4. Development time of R⁺ (black) and R⁻ (light gray) progeny, expressed as the cumulative proportion emerged. **(A)** On average, R⁺ females emerged 2.26 days earlier than R⁻ females ($P < 0.0001$). **(B)** On average, R⁺ males emerged 1.32 days earlier than R⁻ males ($P < 0.03$).

The speed at which *Rickettsia* moved through the whitefly population and the apparent differences in the phenotype of the association in Israel and the United States suggest an especially dynamic interaction between *Rickettsia* and their whitefly hosts.

In general, new associations of inherited symbionts and hosts may result in rapid evolution of both partners and phenotypic shifts that optimize the symbiosis (29). Both the transformation of a host population with a new symbiont infection and the subsequent coevolution of the symbiotic partners are important sources of evolutionary novelty and adaptation for insect hosts. In hosts that are invasive agricultural pests or disease vectors, symbiont infection may both increase the speed at which they adapt to their new environment and exacerbate their influence (1). Clarifying the role of symbiotic microbes may offer insights for ameliorating pest invasiveness or impact (30, 31).

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Supporting Online Material

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Materials and Methods

SOM Text

Table S1

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01. Mathematics and Computational Science, 02. Material Science and Engineering, 03. Communication and Informational Processing, 04. Computer and Software, 05. Civil Engineering, 06. Manufacturing and Automation, 07. Marine Biology and Marine Ecology, 08. Micro-nano Photon-electronics and its Devices, 09. Biophotonics, X-ray Optics, 10. Molecular Biology, Molecular Target based Anticancer Drugs, Cancer Biology, Stem Cell and Immunity Allergic Reaction, 11. Comparative Literature, Western Philosophy, 12. Economics, 13. Management, 14. Industrial Design, Animation Design, Environmental Design

Qualifications

1. Have an earned doctorate in a closely related discipline with a track record of extraordinary accomplishment in teaching, research / scholarship, and professional service judged by peers to be outstanding;
2. Have assistant-professor or above academic experience in world-renowned universities;
3. Have been recognized nationally or internationally for the importance of their achievements and are expected to bring distinction to SZU and serve as a key contributor to achieving its strategic goal of becoming a World-Class university.
4. Have great academic potential and be able to anticipate the development tendency of their field.
5. Have a strong organizing and communicating ability to lead their research team to reach world leading level.

Salary/Benefits

Salary and benefits are very competitive; yearly salary starting from 100,000 to 200,000 US Dollars and will be commensurate with qualifications and experience.

To Apply

Interested candidates should submit all application documents to the below contacts or submit all information to <http://szuhr.szu.edu.cn>. For more information please visit <http://www.szu.edu.cn/szu2007/indexe.asp>

Contact

Ms. Samantha ZENG, (zengsa@szu.edu.cn, 0086-755-26732890) or Ms. Yun LI (liyun@szu.edu.cn, 0086-755-26536111)



Faculty position in Immunology at the University of Alaska Fairbanks

The Institute of Arctic Biology (<http://www.iab.uaf.edu/>) and the Department of Biology and Wildlife (<http://www.bw.uaf.edu/>) at the University of Alaska Fairbanks seek applicants for a **tenure-track faculty position in immunology** at the level of Assistant or Associate Professor. Applicants must possess a doctorate degree in immunology or related field. Postdoctoral research and teaching experience are preferred. Appointment at the Associate Professor level requires a demonstrated record of extramural funding and supervision of graduate students.

Responsibilities will include advising graduate students and teaching undergraduate and graduate courses in immunology, as well as specialized offerings to support the graduate program. The successful candidate will also be an Alaska IDeA Network of Biomedical Research Excellence (INBRE) (<http://www.alaska.edu/inbre/>) investigator, and is expected to contribute to the INBRE research focus areas of infectious disease, toxicology or basic cell biology. The Alaska INBRE program offers an attractive research startup package and other forms of support for research and outreach.

Applications must be completed online (<https://www.uakjobs.com/>). Review of applications will begin after **May 30, 2011**. Questions can be addressed to **Dr. Jonathan Runstadler** (jarunstadler@alaska.edu).

The University of Alaska is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.

Burnett School of Biomedical Sciences College of Medicine

Scientist/Administrator to serve as Associate Director

Burnett School of Biomedical Sciences, College of Medicine seeks an Associate Director to provide leadership to its research enterprise. The school is expanding its research programs in Cancer, Cardiovascular and Metabolic, Neurodegenerative and Infectious diseases into the new 198,000 sq.ft. Burnett Biomedical Science Building. The school will be recruiting more than a dozen new faculty within the next few years. The Burnett School has over 2400 majors and 16,000 students are enrolled in the courses taught by the school faculty. The school offers MS and PhD degrees and MS/MBA and PhD/MBA programs.

The Associate Director is expected to maintain a highly funded research program in any of the four focus areas of the school and provide leadership to the entire research enterprise and graduate programs of the school. The successful candidate must hold an earned doctorate in a discipline appropriate to the school and must have a distinguished record of scholarly accomplishment meriting appointment with tenure at the rank of professor in the school.

A leadership role in recruiting new faculty will be expected. State of the art research facilities including shared core instrumentation and a large transgenic animal facility are available in the Burnett School. UCF the nation's third largest university, ranked third in innovation and patents, is located in the great metropolitan area of Orlando. The Burnett Biomedical Science building is located in the new Health Science campus at Lake Nona that is home also for the new College of Medicine, Sanford-Burnham Medical Research Institute, Nemours Children's Hospital and VA hospital, the beginnings of an exciting major medical city in Central Florida. Further information is available at <http://www.biomed.ucf.edu>

Please submit nominations or a CV and a list of at least four references to pk@mail.ucf.edu

The University of Central Florida is an equal opportunity, equal access, and affirmative action employer. As a member of the Florida State University System, all application materials and selection procedures are available for public review.



THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY

Division of Life Science Faculty Positions

HKUST is a publicly-funded research university with strong graduate programs. The Division of Life Science (<http://life-sci.ust.hk/>) was established recently through the integration of the Departments of Biochemistry and Biology with the goal of raising life science teaching and research at HKUST to a new level of excellence. The Division currently has 29 faculty members representing a broad range of significant expertise including cellular regulation and signalling, cancer biology, developmental biology, molecular and cellular neuroscience, macromolecular structure and function, marine and environmental science, biotechnology and medicinal biochemistry, etc. This newly formed Division is expected to grow substantially in terms of academic staff, programs, resources and facilities in the next five years.

Applications are invited for two tenure-track positions at the Assistant Professor and Associate Professor levels, specifically in the areas of "Developmental Biology" using model organisms such as fly, worm and zebrafish, as well as "Stem Cell Biology" using embryonic or adult stem cells. Applicants of exceptional qualifications and prominence at the full professor rank in areas that complement and enhance existing strengths of the Division will also be considered.

Applicants are expected to establish an internationally competitive independent research program. Teaching responsibilities include undergraduate and postgraduate courses.

Starting salary will be commensurate with qualifications and experience. Fringe benefits including medical/dental benefits and annual leave will be provided. Housing benefits will also be provided where applicable. Initial appointment will normally be on a three-year contract, renewable subject to mutual agreement. A gratuity will be payable upon successful completion of contract.

Applications indicating the areas of expertise, together with a curriculum vitae, a short statement on research interests and the names and addresses of three referees should be sent to the **Chair of the Life Science Search and Appointments Committee (Prof. Mingjie Zhang)**, c/o The Secretary of LIFS S&A Committee, Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong (email: lifssearch@ust.hk) before **15 May 2011**. Review of applications will start in May 2011 and will continue until the positions are filled.

(Information provided by applicants will be used for recruitment and other employment-related purposes.)



中国科学院北京基因组研究所

BEIJING INSTITUTE OF GENOMICS, CHINESE ACADEMY OF SCIENCES

Faculty positions in Computational Biology, Genomics and Systems Biology

Beijing Institute of Genomics, Chinese Academy of Sciences (CAS),
Beijing, China

Beijing Institute of Genomics (BIG) invites applications for faculty positions in the following areas of research:

1. Computational biology that encompasses the development of computational tools and the applications of such tools to biological questions. Algorithm, software and database developments are encouraged.
2. Large-scale genome sequencing, assembly, annotation and analysis as well as new sequencing technology and instrument R&D.
3. Systems biology, including the analysis of transcriptome, epigenome and proteome, that addresses fundamental biological, medical and agricultural questions.
4. Synthetic biology that focuses on the applications of genomics to questions of biosynthesis, bio-energy, and genomic engineering.

Candidates must hold a Ph.D. degree and 3-4 years of post-Ph.D. training in order to qualify for the "100 talent" program of CAS. BIG has fully-equipped platforms for high throughput DNA sequencing, rapid genotyping, and high-performance computing. New faculty members will join an interactive, interdisciplinary community of scientists at CAS after BIG moves into the brand new facility in Beijing Olympic Village in 2012. BIG offers a competitive start-up package that includes research funding, supporting staff, and family relocation benefits.

Inquiries in subfields of genomics not listed above are welcome. Candidates should prepare an application that includes a Curriculum Vitae, a description of past and proposed future research (3-7 pages) and copies of three representative publications. Three signed letters of reference in PDF format should be arranged by the candidate and sent directly by the senders. All documents should be sent to job@big.ac.cn.



Georgia State University

Three faculty positions in Diagnostics and Therapeutics Research

As part of GSU's Second Century Initiative (2CI) to add 100 faculty members over the next five years in selected areas, the College of Arts and Sciences plans to fill a cluster of three tenure-track faculty positions (rank open) within a year in the areas of chemical probe development (chemical biology), biomarkers, and chemosensors, pending budgetary approval. Emphasis will be on recruiting established investigators with external funding. The planned faculty recruitment is part of an overall effort to establish a Center for Diagnostics and Therapeutics at GSU, and along the strategic directions of Georgia Research Alliance and Georgia Cancer Coalition, two major organizations providing support to biomedical research in Georgia. Each appointment will be made to an academic department in accordance to the individual's degree and area of expertise and will be affiliated with the Center. This Center is housed in the new Petit Science building with 350,000 square feet of space for interdisciplinary research and teaching activities and world-class facilities. The same building also houses the Departments of Biology and Chemistry, the Neuroscience Institute, Center for Biotechnology and Drug Design, an animal facility, a BSL4 lab and several BSL3 labs, Center for Viral Immunology, as well as the Molecular Basis of Disease Program and the Brains and Behavior Program for optimal interactions and collaborations.

Successful candidates must have a Ph.D., M.D. or equivalent degree and demonstrate the ability to establish a vigorous, externally funded research program and to mentor Ph.D. graduate students. Excellence in teaching at both undergraduate and graduate levels is expected. Candidates at senior levels must have strong track records in research and teaching excellence, and in securing external funding. Applicants should submit a CV, detailed research plan(s), a statement of teaching philosophy, and a list of at least three references to: **Dr. Binghe Wang, Diagnostics Faculty Search Committee, Department of Chemistry, Georgia State University, P.O. Box 4098, Atlanta, GA 30302-4098.** Application processing will begin immediately and search will remain open until filled.

Georgia State University is a Research University of the University System of Georgia. Offers of employment will be conditional upon background verification.

URL: <http://chemistry.gsu.edu>

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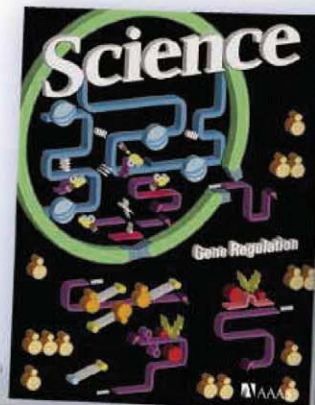
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DEPARTMENT OF BIOLOGY

Full Professorships

The University of York is consistently ranked in the top ten universities in the UK and in the top 100 universities in the world. Named University of the Year in the 2010 Times Higher Education Awards, virtually all of our research is internationally recognised and we attracted over £200 million of funding last year.

The Department of Biology is an expanding and thriving research community of the highest international calibre. We cover the spectrum of contemporary biological sciences with no internal barriers while encouraging collaborations and external links. Biology at York ranked first-equal among UK broad-spectrum biology departments for research judged "world-leading" by the 2008 UK RAE and has recently featured as one of only seven UK departments included in the Times Higher Education top 50 World Ranking of Life Science Departments for 2010.

We are now commencing a major new initiative to recruit international leaders in key strategic areas in the Department. Our aim is to expand our research activity and develop new cross-disciplinary links with other science departments at York (e.g. Chemistry, Physics) and the Hull York Medical School (particularly the Centre for Immunology and Infection). We wish to recruit world leaders in Plant Biology, Molecular Biophysics, and Mammalian Cell Biology to complement and expand our existing strengths.

Candidates for all posts should be outstanding scientists and leaders with excellent track records, well-established reputations in their specific field and proven ability in obtaining significant external grant support. Applicants should possess a strong commitment to the promotion of teaching and learning, and will also be expected to provide leadership in research supervision, management and administration.

Chair in Plant Biology (Ref: UoY01215)

Applications will be considered from across the discipline reflecting the breadth of expertise in the Department from ecology to cell signalling and metabolic engineering. Closing date: 16 May 2011. Interview date: 21 June 2011.

Chair in Mammalian Cell Biology (Ref: UoY01217)

Candidates with interests in cell signalling, cytoskeletal interactions, ageing and apoptosis are encouraged, although other areas of expertise will also be actively considered. Closing date: 3 June 2011. Interview date: 19 July 2011.

Chair in Molecular Biophysics (Ref: UoY01216)

Applicants should be addressing major biomedical/biological questions using ensemble solution biophysical methods and/or single-molecule detection methods, preferably combining in vitro with in vivo measurements. Closing date for this post will be published online only in subsequent months.

Salary will be commensurate with experience on the professorial scale.

For further information and to apply on-line, please visit our website: <http://www.york.ac.uk/jobs/>
Alternatively contact HR Services on 01904 324835 quoting the appropriate reference number.

The University of York is committed to promoting equality and diversity.



www.york.ac.uk



Director Gastrointestinal Immunology Program

The Department of Medicine at the Georgetown University Medical Center is seeking a dynamic founding director of the Gastrointestinal Immunology Program. The ideal candidate will have an MD or MD/PhD degree, and an established track record of NIH – supported research of gastrointestinal immunology. The candidate will establish and lead a translational effort in immunology to be integrated with clinical and basic science investigators within the Georgetown scientific community, and the Georgetown - Howard Universities Center for Clinical and Translational Science. A clinical focus and the ability to collaborate with other investigators will be advantageous. Appointment will be at the Professor or Associate Professor level.

Please send curriculum vitae to **Tolise Miles** at tcm9@georgetown.edu, and contact Ms. Miles for questions via e-mail or by phone at (202) 444-7309.

Georgetown University is an Affirmative Action/Equal Opportunity Employer.



FACULTY POSITION IN BIOCHEMISTRY

The University of Alaska Fairbanks Department of Chemistry and Biochemistry, the NIH supported Alaska IDEA Network for Biomedical Research Excellence (INBRE) and the Institute of Arctic Biology (IAB) invite applications for a tenure track position at the Assistant Professor level in Infectious Disease, Toxicology or other related areas. The candidate will join a growing group of INBRE sponsored research faculty at the University of Alaska Fairbanks focused on molecular mechanisms in the thematic area of Environmental Agents of Disease. Current INBRE faculty are actively pursuing research into the emergence, re-emergence and spread of infectious agents, Zoonotic diseases, Contaminants and Molecular Toxicology. Competitive startup and funding is available. This position is part of the continuing growth in biomedical research at the University of Alaska and coincides with the construction of a new Biological Science Research and Teaching Facility on the Fairbanks campus. Duties of this position include the development of a nationally recognized competitive research program, participating in the Alaska INBRE program, and mentoring of graduate and undergraduate students in research projects. Teaching duties include undergraduate and graduate courses in Chemistry, Membrane Biochemistry, Infectious Disease, Toxicology or related areas. A Ph.D. in Biochemistry or related discipline is required, postdoctoral experience is highly preferred. Please visit www.uaf.edu/chem for information on the Department of Chemistry and Biochemistry, <http://www.alaska.edu/inbre/> for information on the Alaska INBRE program at the University of Alaska Fairbanks or <http://www.iab.uaf.edu/> for the Institute of Arctic Biology.

To apply, complete an online application including; a cover letter, at least three references, a CV, a statement of proposed research, and a statement of teaching philosophy and student mentoring. This online application may be found at <http://www.uakjobs.com/applicants/Central?quickFind=73527>. Please send graduate transcripts to **Marvin K. Schulte c/o Margo Griffith, Chair Biochemistry Search Committee, Department of Chemistry, University of Alaska Fairbanks, PO Box 757000 Fairbanks, AK 99775**. Review of applications begins on **April 22, 2011**.

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Position in CLINICAL MOLECULAR DIAGNOSTICS

The Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, invites applications for a full-time academic position in the Division of Diagnostic Molecular Genetics. This position includes clinical responsibilities in Molecular Diagnostics and Cytogenetics. The individual will have responsibilities that include case interpretation, technical oversight and monitoring of compliance with OLA/CCMG requirements and guidelines as well as new test development and implementation. The Cytogenetics Laboratory provides diagnostic services for constitutional cytogenetic anomalies as part of a CCMG-accredited comprehensive training and service facility. Candidates should possess the vision and expertise necessary to integrate cutting-edge clinical service into an environment rich in both research and education. The clinical laboratory will provide state-of-the-art diagnostics using genotyping, high throughput and Next Gen sequencing, array-based CGH, and a variety of in situ hybridization methodologies, and will develop and bring into clinical practice novel applications of these and other, newly-emergent diagnostic technologies. The candidate will be expected to develop a program of clinical, translational and/or basic research and to participate in education activities, with an emphasis on the training of residents and fellows in personalized medicine.

This is a challenging opportunity requiring excellent communication, analytical, organizational and interpersonal skills, as well as the ability to lead and guide the laboratory team in the efficient delivery of molecular diagnostic services as well as to interact with clinicians in a tertiary/quaternary medical center.

Minimum qualifications include an MD or PhD in Genetics or a related field with certification or eligibility for certification by the Canadian College of Medical Geneticists or the American Board of Molecular Genetics. Preference will be given to candidates with demonstrated relevant clinical and academic experience. The individual we seek would be expected to qualify for appointment at the Assistant Professor Level.

Please submit curriculum vitae which includes the names of three references by **May 6, 2011** to: **Dr. Rita Kandel, Chief, Dept. of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, ON M5G 1X5.**

In accordance with Canadian and immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. Mount Sinai Hospital respects, appreciates, and encourages diversity. Mount Sinai Hospital is an Equal Opportunity Employer.

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From the journal Science



Research Fellow Positions at Suzhou Institute of Biomedical Engineering and Technology (SIBET), Chinese Academy of Sciences (CAS) Suzhou, Jiangsu, China

SIBET is situated in Suzhou, an ancient city near Shanghai in China. SIBET is focusing on the breakthrough of core technologies in detection based on spectroscopy, nuclear medical detectors, micro-nano sensors and medical semiconductor laser by integrating high technology in fields of biology, photoelectricity, nuclear medicine and computer. SIBET is dedicated to develop mature products in the medical instrumentation, medical materials and biomedical reagents to meet the demands of public health and domestic medical industry development. Currently, the R&D fields include medical laser, biomedical measurement, medical imaging, biomedical electronics, biomaterials and biomedical reagents.

We are seeking candidates for research fellow positions at Associate Professor/Professor levels to work in the fields of (1) medical laser, (2) medical imaging, (3) clinical diagnosis and analysis, (4) biomedical electronics, (5) biomedical reagents, (6) artificial organ and (7) biological and medical materials.

Generous start-up packages to develop successful labs and competitive salaries and benefits will be provided.

Candidates should have a Ph.D., an outstanding track record of scientific productivity, the ability to manage labs and research projects independently, and excellent communication skills in Chinese and English.

Send curriculum vitae, a statement of research interests and future plans, and a list of three references to:

Prof. Xuan Ming,

President, Suzhou Institute of Biomedical Engineering and Technology (SIBET)

Human Resource Department of SIBET

88 Keling Road, SND, Suzhou 215163, China

Tel: 86-512-69588025; Fax: 86-512-69588088

Email: talent@sibet.ac.cn



Professorships and Chair Professorships at Shaanxi Normal University, China

陕西师范大学诚聘英才

Founded in 1944 and located in The World-Famous Historical City – Xi'an, Shaanxi Normal University is one of China's key normal universities under the direct supervision of the Ministry of Education. The university is seeking individuals with outstanding scientific credentials for Recruitment Program of Global Experts, Chang Jiang Scholars Program, One hundred talents Plan of Shaanxi Province and Qu Jiang Scholars Program of our university, which are designed for the recruitment at the level of professor, associate professor and chair professor.

Qualifications

Applicants are expected to have remarkable academic achievements and demonstrated capacity to lead an academic team to keep a competitive edge in frontier areas. Successful candidates for the professorship will be expected to undertake full-time teaching and research, and those for the chair professorship to work part-time (two months minimum/year).

Salary and Housing Allowances

Highly competitive salaries, housing allowances and initial research startup funds will be provided depending on nature of the discipline and qualifications of the successful applicants.

Application Documents

Applications should include a résumé with a list of publications, a concise statement of research and teaching interests, and the names and addresses (including e-mail) of at least three referees.

You are welcome to click on the university website at <http://rsc.snnu.edu.cn/zhaopin.asp> for further details.

Applications should be submitted electronically to:

Mrs. Wu Jinfeng or Mr. Yang Yuanzheng

E-mail address: rcb@snnu.edu.cn

Tel: 86-29-85310456, 86-29-85310455

Fax: 86-29-85310359

CHAIR

DEPARTMENT OF ANATOMY

THE UNIVERSITY OF MISSISSIPPI
MEDICAL CENTER • JACKSON

The University of Mississippi Medical Center (UMMC) seeks a dynamic person who can lead our Department of Anatomy and foster a contemporary neurobiology research program. The department is noted for excellence in teaching and significant neurobiology research. The department has responsibility for teaching four first-year medical courses and two first-year dental courses. Five of these courses have major laboratory components. Plans are underway to increase the size of the department by adding several faculty members with primary emphasis in neurobiology research. This emphasis will be highlighted by changing the department's name to "Neurobiology and Anatomical Sciences."

The University of Mississippi School of Medicine is committed to recruiting and hiring leaders who are "future oriented" and possess the following characteristics: business and administrative experience; institutional orientation; strong communication skills; ability to build and lead a team; and a results orientation. Candidates should have a strong history of successful, funded research in neurobiology, demonstrate an interest in high educational standards and abilities to administer and nurture a diverse basic science department, including attracting and retaining successful research and teaching personnel. The successful candidate must have a PhD or equivalent, and will receive a tenure track academic appointment at the level of professor with a competitive salary as appropriate to his or her record of accomplishment.

Interested candidates should submit a curriculum vitae and a cover letter that includes (1) a description of leadership and administrative experience, including a general plan for administering a contemporary anatomy department; (2) description of teaching experience and philosophy; and (3) a statement of research interests that includes current and recent research support, as well as future research plans.

Dr. Patrick Smith
Associate Dean, Office of Faculty Affairs
University of Mississippi School of Medicine
2500 North State Street
Jackson, Mississippi 39216-4505
posmith@umc.edu
601-984-4683 (Fax)

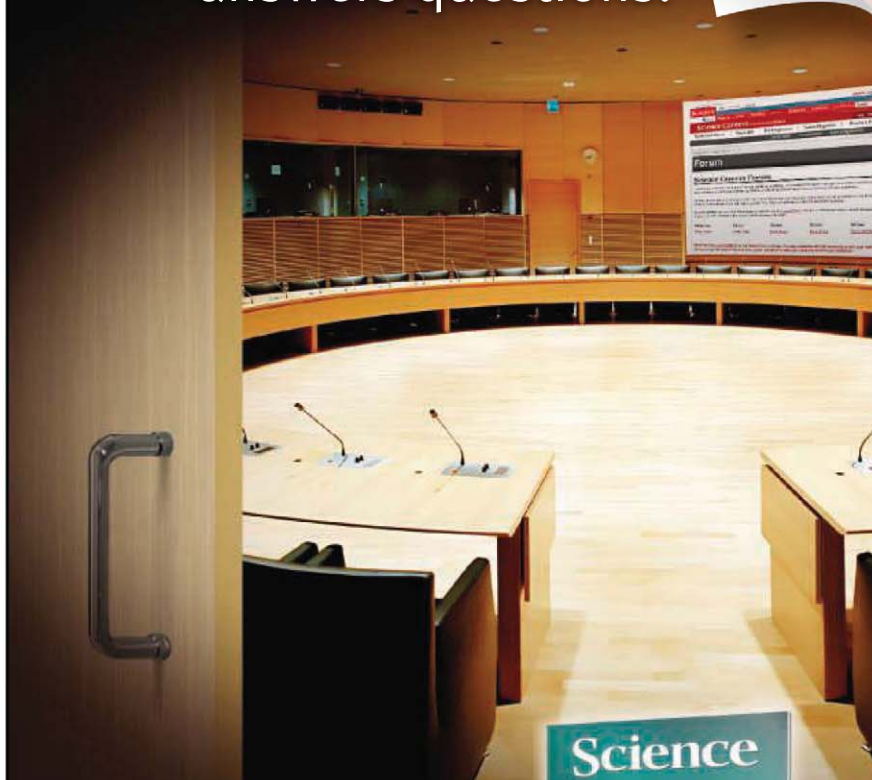


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AWARDS



HUMAN FRONTIER SCIENCE PROGRAM (HFSP)

CALL FOR NOMINATIONS FOR THE 2012 HFSP NAKASONE AWARD

In keeping with its mission to stimulate innovative international research, HFSP invites nominations for an annual award highlighting frontier contributions in the life sciences. Typically these will be conceptual breakthroughs for investigating the complex mechanisms of living organisms which have important consequences for scientists throughout the world. Both theoretical and methodological contributions are eligible. This award recognizes the vision of former Prime Minister Nakasone of Japan in the creation of HFSP.

The winner of the 2011 award was Michael Elowitz of the California Institute of Technology, for his pioneering work on gene expression noise.

The competition is open; it is not limited to HFSP awardees and there is no age limit for candidates. However the jury will pay particular attention to recent breakthroughs by younger scientists. Nominations should be made before Wednesday April 27th by submitting the standard one-page nomination form (<http://bit.ly/e2hSlS>) and the nominee's CV. The selection will be made by the HFSP Council of Scientists at their meeting in June 2011.

The awardee will receive an unrestricted research grant of 10,000 USD, a commemorative medal and will be expected to deliver a plenary lecture at the 2012 HFSP Awardees Meeting (in Korea, July 1-4, 2012).

Please see our web site www.hfsp.org for further information

HFSP, 12 quai Saint-Jean, 67080 STRASBOURG Cedex, FRANCE

Cellular Immunologist and Host Defense Researcher University of Texas Medical Branch, Galveston

The Department of Pathology, University of Texas Medical Branch, Galveston, is seeking applications for an Associate or full professor to lead and expand the established Immunology Program.

The Immunology Program will include at least fifteen, full-time faculty members, whose research is focused on infectious, immunological, metabolic, nutritional, and gestational diseases in which innate and adaptive immunity play a key role in infection control or disease pathogenesis. Members of the Program have collaborative projects and active interactions with University scientists in several departments and in the Galveston National Laboratory.

The successful applicant will have an extensive body of influential scientific publications, experience in mentoring successful research careers, an established international reputation in the field of Cellular Immunology, Host Defense, or Pathobiology, and an active research funding program which employs cutting-edge technologies and immunologically relevant models of infectious diseases.

Experience in developing research priorities, defining and pursuing research opportunities, and building collaborative research initiatives is essential, as is familiarity with research-based postgraduate and student research programs.

Applicants should send a letter of interest, statement of current and future research objectives, and curriculum vitae to:

Lynn Soong, M.D., Ph.D.
University of Texas Medical Branch
301 University Boulevard
Galveston, Texas 77555-1070 USA
Email: lysoong@utmb.edu

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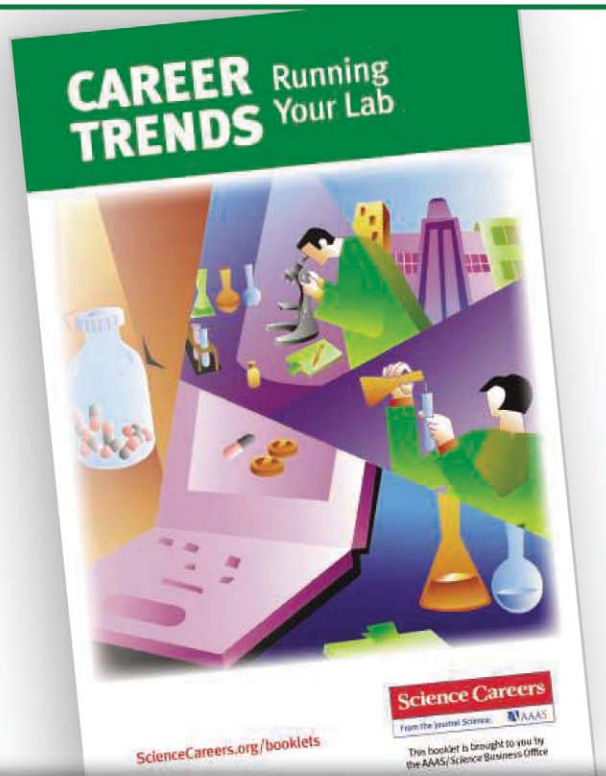
Hematological and Cardiovascular Research

Soochow University is a premier research university in hematology and cardiovascular biology in China with internationally recognized programs in thrombosis, hemostasis, proteases and leukemia. The University is located in Suzhou, one of the most desired cities in China. With newly established Cyrus Tang Hematology Center and substantial funding from Jiangsu government, a major expansion of its research programs is under way at a new campus.

We are seeking candidates at Associate Professor and Professor levels to work in the fields of (1) **arthrosclerosis, hemostasis, thrombosis, and vascular biology**, (2) **malignant hematological disorders**, and (3) **hematopoietic stem cells**. Candidates with strong background in genetics, structural biology, and animal models are encouraged to apply. We offer state-of-the-art core facilities, large collections of clinical samples, and a new animal center.

Candidates should have a Ph.D. or M.D., outstanding track records of scientific productivity, and the ability to develop independent research projects. Successful candidates will be provided with generous start-up and relocation packages and competitive salaries/benefits.

Send your curriculum vitae, a statement of research interests and future plans, and a list of three references to **Ms. Qiaoqiao Chen** (cqq@suda.edu.cn) at Dushu Lake Campus, Soochow University, 199 Ren-Ai Road, Suzhou Industrial Park, Suzhou 215123, Jiangsu, China.



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**OPEN RANK FACULTY POSITION
Earth and Environmental Sciences**

The Department of Geological Sciences at Michigan State University invites applications from outstanding candidates for a tenure-track faculty position at the **ASSOCIATE** or **FULL PROFESSOR** level. Our intent is to recruit a colleague who will take advantage of projected areas of strategic investment in Earth and Environmental Sciences, complement existing Department strengths, and link to interdisciplinary programs across the University. Applicants are expected to be leaders in their fields and must have well-funded research programs and an exemplary record of scholarship. Interested candidates are welcome to contact the Department Chair, **Dr. David Hyndman** at telephone: 517-355-4626 or e-mail: eeshire@msu.edu. The early phase of the application process can be handled in a confidential manner. Review of applications will begin on June 15, but the position will remain open until filled. For more information, visit website: <http://glg.msu.edu/excellencehire.html>.

MSU is an Affirmative Action/Equal Opportunity Employer, committed to achieving excellence through cultural diversity. We actively encourage applications and/or nominations of women, persons of color, veterans, and persons with disabilities.

**DIRECTOR OF ELECTRON MICROSCOPY
Yale University School of Medicine
Center for Cellular and Molecular Imaging
Website: <http://medicine.yale.edu/cellbio/ccmi/ccmi/index.aspx>**

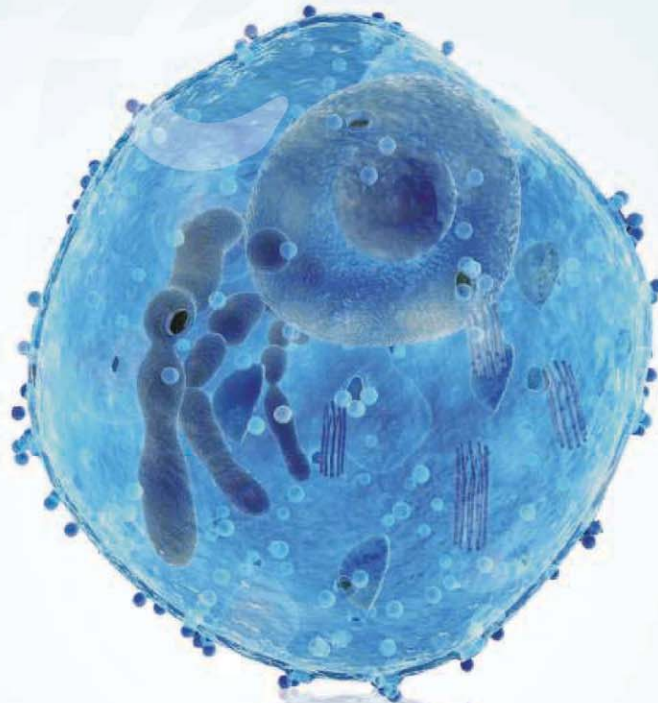
A Director of Electron Microscopy is sought for the primary EM core at Yale University School of Medicine. The core provides faculty members, postdoctoral fellows, and students from across the university with access to state-of-the-art expertise and instrumentation for studies that require standard electron microscopy, cryoelectron microscopy, and tomography. The ideal candidate for Director will develop collaborative EM research with Yale laboratories, prioritizing these collaborations based on scientific feasibility and promise. Other duties include methods development and providing advice and assistance to a wide range of users as well as to the skilled four-member staff who carry out routine work at the facility. The individual should be capable of multitasking and should enjoy working with other people. Good communication skills are essential. Ph.D. in biology, physics/materials science, or a related field and experience in electron microscopy and image processing are required. Applications, including cover letter, curriculum vitae, and names of three referees should be sent electronically to **James Slattery**, e-mail: james.slattery@yale.edu and are due by May 6, 2011. *Applications from, or nominations of, women and minority scientists are encouraged. Yale is an Affirmative Action/Equal Opportunity Employer.*

**SENIOR POSTDOCTORAL ASSOCIATE
Boston University**

Required expertise in transgenic rats, ultrasound molecular imaging, arterial stiffness analysis, and sonoporation, FACS, MoFlo, confocal microscopy. M.D. or Ph.D., \$40,000/year. Send curriculum vitae to **Nancy Clinton**, ncClinton@bu.edu. *Equal Opportunity Employer/Affirmative Action.*

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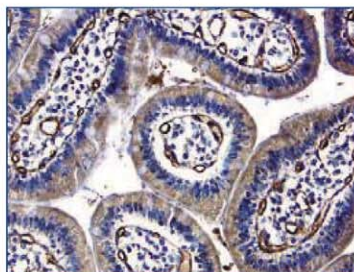
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R&D Systems Specialized Tools for Cancer Research

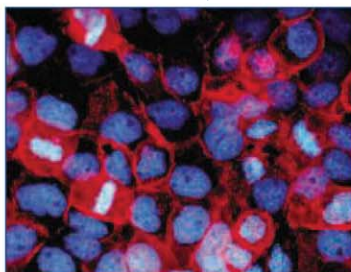
Cancer is a disease of cellular dysfunction involving a range of biological activities that promote unregulated proliferation. To help advance the understanding of this complex disease, R&D Systems offers an expansive selection of quality products, including **high performance antibodies, proteins, ELISAs, & arrays**, for investigating various cancer-related factors. Every product from R&D Systems undergoes rigorous quality control testing to ensure industry leading reliability.

Angiogenesis



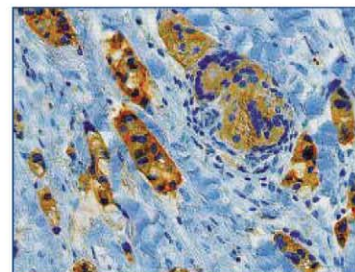
Angiopoietin-2 (Catalog # AF623)
Human Gastrointestinal Cancer Tissue

Epithelial to Mesenchymal Transition



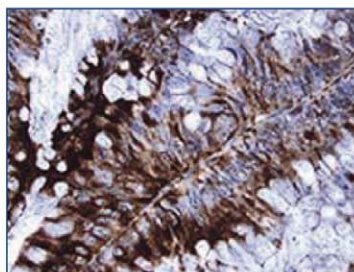
E-Cadherin (Catalog # AF648)
Human Epidermoid Carcinoma Cells

Cancer Biomarkers



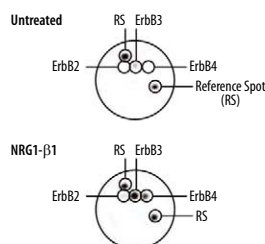
BRCA1 (Catalog # MAB22101)
Human Breast Cancer Tissue

Inflammation



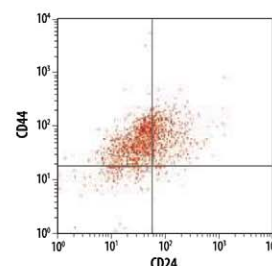
EMAP-II (Catalog # AF1910)
Human Colon Cancer Tissue

Growth Factor/Receptor Signaling



Proteome Profiler™ 96 Phospho-RTK Antibody
Array (Catalog # ARZ001)
MDA-MB-453 Cell Line

Cancer Stem Cells



MagCelect CD24-CD44⁺ Breast Cancer
Stem Cell Isolation Kit (Catalog # MAGH111)
MCF-7 Cell Line

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